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# Evaluation of Airborne Exposure Limits for VX: Worker and General Population Exposure Criteria

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Prepared For

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13. ABSTRACT *(Maximum 200 words)* Existing occupational airborne exposure limits (now referred to as "worker population limits" or WPLs) and general population airborne exposure limits (now referred to as "general population limits" or GPLs) were reviewed and recalculated using current risk assessment methods and two sets of data not considered in previous estimates. These "newer" data resulted in estimated WPLs and GPLs lower than existing values. However, the quality of both sets of data was such that there was not sufficient confidence to select either as a critical study. Overall, the quality and quantity of the data for VX are less than desirable. In addition, no chronic studies have been done. Given this, it was decided to develop the exposure limits for VX relative to those for GB. A potency ratio of 10 was selected, based upon relative potencies of GB and VX in producing miosis. (It was noted, however, that unlike GB, VX vapor is a percutaneous hazard, and the relative potency ratio for threshold percutaneous effects is about 100.) Based upon the miosis potency ratio, the existing WPL for VX (0.00001 $mg/m^3$) was deemed adequately protective. However, the existing GPL was not, and a concentration of 0.0000003 $mg/m^3$ is recommended. The existing immediately dangerous to life or health (IDLH) exposure level for occupational workers was recalculated relative to that for GB. A value of 0.01 $mg/m^3$ is recommended. A short-term exposure limit (STEL), for workers, was developed relative to that for GB. The recommended value is 0.00004 $mg/m^3$, for up to four 15-minute exposures per day. Similarly, relative to those for GB, acute exposure guideline levels (AEGL-1) were developed for the general population for exposure durations of 30 minutes, 60 minutes and four hours. The recommended concentrations are 0.00024 $mg/m^3$, 0.00012 $mg/m^3$, and 0.00003 $mg/m^3$, respectively.

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## EXECUTIVE SUMMARY

### PURPOSE

The purpose of this document is fourfold:

(1) To review: a) the existing VX AELs for the workforce and general population and b) any relevant data which have become available since the existing estimates were first derived. (2) To apply currently accepted risk assessment approaches to the data most relevant to human exposure scenarios, in order to: a) ascertain whether the existing exposure limits are acceptable by current standards and/or b) derive new ones, if necessary. (3) To estimate long-term (*e.g.*, Worker Population Limit or WPL and General Population Limit or GPL) and acute [*e.g.*, "immediately dangerous to life and health" (IDLH)] exposure guidelines for VX. (4) To derive exposure criteria for "short-term exposure limits" (STEL) and "acute exposure guideline level one" (AEGL-1), which do not currently exist.

### DISCUSSION

(1) VX [S-(2-diisopropylaminoethyl) O-ethyl methylphosphonothiolate] is a very potent organophosphorous anticholinesterase (anti-ChE) compound of the type commonly referred to as a "nerve gas". Small quantities of such chemical warfare (CW) agents or agent by-products are used by various military and contract laboratories for defensive research purposes and verification of Chemical Weapons Convention compliance. Although bulk quantities are no longer manufactured in the United States, they currently exist in military stockpiles where they await eventual destruction.

(2) People whose work environment may contain chemical weapons agents, whether in storage depots and demilitarization facilities, laboratory research, verification of the Chemical Weapons Convention, remediation and decontamination, or emergency response operations, face potential risk of accidental exposure to these materials. This risk is also shared by the general population in communities surrounding areas in which chemical agents are stored, transported, or processed for disposal. In addition, chemical weapons, whether in foreign or domestic stockpiles, still represent current military threats and potential terrorist targets. The most likely route of systemic exposure is by inhalation, but effects from airborne vapor also include the direct local effects of chemical agent vapor upon the eyes, respiratory tract, and skin.

(3) The existing airborne exposure limits (AELs ) for VX, that were promulgated by the Centers for Disease Control (CDC) (DHHS, 1988), were originally put forward by McNamara *et al*. in 1973. They were based upon very limited data, none of which was for vapor inhalation exposure to VX. Since that time two sets of salient VX inhalation data (Bramwell *et al.*, 1963; Crook *et al.*, 1983) have become available.

## FINDINGS AND CONCLUSIONS

(1) There are no chronic studies of VX vapor exposure by inhalation or any other route. The existing airborne exposure limits for VX were based upon blood cholinesterase (ChE) inhibition, relative to GB, for routes of exposure other than inhalation. Current methodology does not support this approach because the critical effects secondary to inhalation exposure—miosis, tight chest, and rhinorrhea, are different from those occurring following other routes of exposure. Moreover, these critical effects occur at dosages lower than those required to produce inhibition of blood ChE. It is also noted that, percutaneous (PC) vapor exposure to VX poses a significantly greater hazard than such exposure to GB. Neither of the "newer" sets of VX inhalation data supports the existing criteria. In fact, the animal study (Crook *et al.*, 1983) indicates effects in animals at concentrations lower than the WPL. However, both studies are flawed, and there is not a high degree of confidence in the reported vapor concentrations in either. It was concluded that these studies should not be ignored, but the quality of the analytical data is too poor to use them as a basis for establishing chronic exposure criteria. The only organophosphonate nerve agent for which there are sufficient data for establishing inhalation exposure criteria is GB. The WPL and GPL criteria derived herein for VX are referenced to those for GB, as recently proposed by Mioduszewski *et al.* (1998). A potency ratio of 10 was selected, based upon effective dosages for miosis (IDA, 1998). [Reutter and Wade (1994) endorsed the existing estimate of 0.09 mg min/$m^3$, for miosis secondary to airborne VX (2-10 minute exposure), but recommended lowering the estimate for GB to 0.5 mg min/$m^3$ (2-10 minute exposure). The COT (1997) suggested that the GB estimate should be higher than that put forward by Reutter and Wade. At the IDA workshop, it was agreed to recommend an estimate of 1 mg min/$m^3$ for GB and to round the estimate for VX to 0.1 mg min/$m^3$, so as not to indicate a level of precision that does not exist.] However, it is noted that airborne VX presents a significant percutaneous vapor hazard, and the potency ratio for threshold percutaneous effects is estimated to be 120. As with all documents such as this, the criteria proposed herein should be re-evaluated as new data become available.

(2) The proposed AELs for VX are shown in the table below. The following points should be carefully noted:

The biological endpoint selected for determining the IDLH estimate includes generalized weakness, signs of systemic V-agent poisoning and less serious effects including miosis, rhinorrhea, periorbital fasciculations, and tightness of the chest. IDLH estimates are limited to acute exposures (up to 30 min).

Exposures above the WPL up to the STEL should be no longer than 15 min, and should not occur more than four times per day. There should be at least 60 minutes between successive exposures in this range. If individuals are exposed to concentrations above the WPL up to the STEL, these exposures must be considered in the 8-hour TWA such that the WPL is not exceeded as a cumulative daily exposure. The developed STEL value is based upon the estimated relative potency of VX as compared with GB, for estimated airborne concentrations associated with "no observable adverse effects" in a human workforce population.

The acute exposure guideline levels limited to discomfort (AEGL-level 1) are estimates for acute (30 min, 1 hr, and 4 hr) exposure scenarios associated with the lowest observable adverse effects (miosis, rhinorrhea and tightness of chest) in humans (general population).

**Recommended Airborne Exposure Limits (AELs) for VX in the Workplace (WPL) and for the General Population (GPL)**

| Criteria ($mg/m^3$) | Application/ Scenario |
|---|---|
| **Occupational** | |
| **0.00001** | WPL (TWA) (8 hr/day, 40 hr wk) |
| **0.01** | IDLH (30 min) |
| **0.00004** | STEL (15 min, 4x/day) |
| **General Population** | |
| **0.0000003** | GPL (TWA) (24 hr/day,7 days/wk) |
| **0.0002** | AEGL-1 (30 min) |
| **0.0001** | AEGL-1 (1 hr) |
| **0.00003** | AEGL-1 (4 hr) |

**IDLH**: Immediately dangerous to life or health.
**STEL**: Short-term exposure limit.
**AEGL-1**: Acute exposure guideline, level 1 (effects limited to discomfort).
**TWA**: Time-weighted average.

## PREFACE

The work described in this report was authorized under MIPR No. 94-237, Chemical Agent Health Criteria Document. The work was started in June 1997 and completed in October 1999.

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This report is approved for public release.

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# EVALUATION OF AIRBORNE EXPOSURE LIMITS FOR VX: WORKER AND GENERAL POPULATION EXPOSURE CRITERIA

## 1. PURPOSE

Airborne exposure limits (AELs) are required for the protection of people who face potential exposure to toxic chemicals. This includes individuals whose work environment may contain chemical warfare agents, whether in storage depots and demilitarization facilities, laboratory research, verification of the Chemical Weapons Convention, remediation and decontamination of contaminated areas, or emergency response operations. This risk is also shared by the general population in communities surrounding the areas where chemical agents are stored, transported or processed for disposal.

In a recent review of the literature on chemical agent toxicity (Reutter and Wade, 1994; COT, 1997), a need to re-evaluate human toxicity estimates for various exposure scenarios was recognized. The purpose of this document is fourfold: (1) To review: a) the existing VX AELs for the workforce and general population and b)any relevant data which have become available since the existing estimates were first derived. (2) To apply currently accepted risk assessment approaches to the data most relevant to human exposure scenarios, in order to: a) ascertain whether the existing exposure limits are acceptable by current standards and/or b) derive new ones, if necessary. (3) To estimate long-term (*e.g.*, AEL Worker Population Limit or WPL and General Population Limit or GPL) and acute (*e.g.*, "immediately dangerous to life and health" (IDLH)) exposure guidelines for VX. (4) To derive exposure criteria for "short-term exposure limits" (STEL) and "acute exposure guideline level one" (AEGL-1), which do not currently exist.

## 2. BACKGROUND

### 2.1 Introduction

VX [O-ethyl S-(2-diisopropyl-aminoethyl) methylphosphonothiolate] is one of the "V"-type agents. The others are VE, VG, VM, VP, and VS. Very little work has been done with the other "V" compounds; VX is considered to be the "standard" for the type (Ward, 1958). It is also known as EA 1701 (US designation) and T 2445 (British designation).

The prototype V-agent was developed as an insecticide and patented by the British in 1955 (Fielding, 1960). However, the material was discovered to be much more potent than was expected from its general chemical structure and was too toxic to humans to use as an insecticide. (By all exposure routes, VX is estimated to be $10^3$ to $10^4$ times more potent than most commercially available organophosphorous insecticides when $LD_{50}$ values are compared (Watson *et al.*, 1992).) Its potential for use as a chemical warfare agent was recognized by the British as early as 1953. Because of its extreme potency, this class of compounds was christened "V

agent"—the "V" standing for venom (Ward, 1958; Fielding, 1960). Like other nerve agents and many insecticides, VX is a liquid organophosphate (OP) ester derivative of phosphoric acid. Its primary effects result from the inhibition of the enzyme acetylcholinesterase (AChE).

As a military agent, VX has been perceived to be primarily a percutaneous (PC) liquid hazard. Liquid VX is several orders of magnitude more potent *percutaneously* than Sarin (GB). However, in vapor or aerosol form, VX can present a considerable inhalation, ocular, and/or dermal hazard. By inhalation, VX is at least twice as potent as GB as a lethal agent, and airborne VX is at least ten times more potent in producing pupillary constriction called miosis (IDA, 1998).[1] GB is not really a percutaneous vapor/aerosol threat. However, airborne VX is a significant percutaneous hazard and is estimated to be at least 100 times more potent than GB (Reutter and Wade, 1994; COT, 1997).

V agents are no longer manufactured in the United States. However, VX is currently stored in military depots/stockpiles, where it awaits eventual demilitarization/destruction. In addition, small quantities are still used by various military and contract laboratories for defense research purposes. All chemical agents, whether in foreign or domestic stockpiles, are considered potential military/terrorist threats.

Determination of exposure criteria depends upon whether the chemical in question is a "threshold" or a "non-threshold" toxicant. VX has been traditionally classified as a "threshold" toxicant, *i.e.*, a minimum dose or level of exposure is necessary to produce toxic responses. This is characteristic of non-carcinogenic chemicals. In contrast, carcinogens are usually considered "non-threshold" toxicants—the response (cancer) can be produced by any amount of agent exposure, no matter how minimal. (Recently, it has been accepted that some carcinogens do have threshold limits.)

Although it has been about 50 years since VX was first synthesized, significant data gaps in its toxicology still exist. This has arisen, in part, from the fact that VX was developed for offensive purposes. Consequently, most of the existing data are for acute exposures of animals to relatively high concentrations. The limited human data consist of acute exposures to relatively low concentrations. The data gaps are further confounded by the fact that most of the database for VX is based on percutaneous exposure to the liquid form. In general, the data for VX are much less complete than those for GB.

## 2.2 Chemical and Physical Properties

The "V" agents contain sulfur, instead of fluorine or cyanide, which is found in the "G" agents, and they are less volatile than the "G" agents are. VX is poorly soluble in water and readily penetrates skin. (See Table 1)

[1] IDA (Institute of Defense Analyses), Report of the Workshop on Chemical Agent Toxicity, held at the Institute for Defense Analyses, May 11-12, unpublished report.

## 2.3 Biological Properties

### 2.3.1 Mechanisms of Action

The most commonly accepted mechanism by which organophosphorus nerve agents (and organophosphorous insecticides) produce their toxic effects is *via* the phosphorylation of the active site of one or more of the ChE enzymes. Inhibition of AChE results in accumulation of the neurotransmitter acetylcholine (ACh) at the cholinergic synapses. This produces overstimulation of the cholinergic nerve fibers and target organs. The effect is uncontrolled, disorganized target tissue responses.

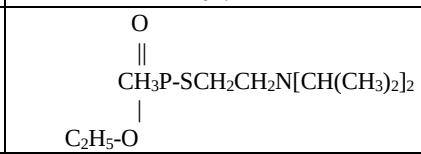
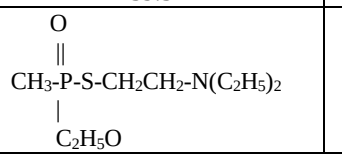
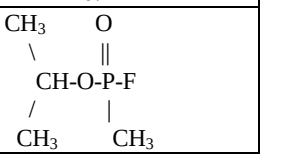
In addition to reacting with ChEs, *in vitro* studies have shown that OPs can react with other components in nerves or in effector organs. Direct effects on the cholinergic receptor or on its phospholipid environment, at both central nervous system (CNS) and peripheral nervous system (PNS) synapses, have been demonstrated by Karczmar (1967), Kuba *et al*. (1974), Gage (1976), Van Meter *et al.* (1978), Baron (1981) and Albuquerque *et al.* (1985). As early as 1931, White and Stedman suggested that, in addition to inhibiting AChE, OP compounds have an effect on the site at which the ACh molecule reacts at the neuromuscular junction. Miquel (1946) suggested that OP compounds react with sites on the muscle, in addition to the enzyme. Similarly, studies by Xavier and Valle (1963) disclosed that Phosdrin, an OP insecticide, was able to affect both the ACh receptor and the ion channel associated with it—without affecting AChE. They also found, using two different methods, that physostigmine and neostigmine, in addition to producing blockade of AChE, potentiated the muscle response to ACh when applied in the presence of complete AChE blockade. The occurrence of these effects *in vivo* has not been confirmed.

The G-agents do not have preferential affinities for the different ChEs of the blood. However, VX preferentially inhibits red blood cell (RBC) AChE, as opposed to the butyrylcholinesterase (BuChE) of the plasma (Feinsilver *et al.*, 1964; Sidell and Groff, 1974). Also, there is significantly more spontaneous reactivation of VX-inhibited RBC-ChE than there is of GB-inhibited RBC-ChE (Sidell and Groff, 1974). (Interestingly, less oxime is required to reactivate VX-inhibited enzyme than GB-inhibited enzyme (Sidell and Groff, 1974).) The $T_{1/2}$ of "aging"—dealkylation of the organophosphate-enzyme complex, is about 48 hours for VX (Sidell and Groff, 1967; 1974). This compares with about two minutes for Soman (GD) (Fleisher and Harris, 1965) and 5 hours for GB (Harris *et al.*, 1967).

The ChEs of the blood are not targets of toxicity. They function more as sinks for anti-ChE agents. Measurements of plasma and erythrocyte ChE activities may constitute very sensitive indices of exposure to anti-ChE agents, but inhibition of these enzymes does not necessarily imply anti-AChE intoxication (Koelle, 1994). Repeated exposure to low doses of ChE inhibitors can produce virtually complete inhibition of the blood ChEs—without clinical signs or symptoms (Freeman *et al.*, 1956; Bertino *et al.*, 1957; Ward, 1958). Conversely, rapid exposure to relatively higher doses can produce marked clinical symptoms in the absence of ChE inhibition (Kimura *et al.*, 1960). Overall, there is poor correlation between dose of anti-ChE and amount of ChE inhibition (Kimura *et al.*, 1960; Sidell and Groff, 1966, 1967, 1974).

It is interesting to note that VX-induced nausea and vomiting and abdominal discomfort may be more prominent and occur with less inhibition of the blood ChEs than has been observed for the G agents (Cullumbine *et al.*, 1954; Sim, 1962). Similarly, rat studies indicate that the brain ChE level at death, following subcutaneous (SC) injection with VX, was 32% of normal. In animals similarly exposed to GB the brain ChE activity at death was only 10% of normal (Harrison *et al.*, 1957). Another difference between VX and the G agents is the time of onset for symptoms. Surprisingly, the signs and symptoms of VX intoxication do not occur as rapidly as they do for the G agents (Rickett *et al.*, 1986).

**Table 1 Chemical and Physical Properties of V Agents Compared with GB**

| | **VX** | **VM** | **GB** |
|---|---|---|---|
| Chemical Name | O-ethyl S-(2-diisopropyl-aminoethyl) methylphosphonothiolate | O-ethyl S-(2-diethyl-aminoethyl) methylphosphonothiolate | Isopropyl methylphosphonofluoridate |
| Molecular Formula | $C_{11}H_{26}N\,O_2PS$ | $C_9H_{22}NO_2PS$ | $C_4H_{10}FO_2P$ |
| Molecular Weight | 267.4 | 239.3 | 140.1 |
| Structure |  |  |  |
| CAS No. | 50782-69-9 | 96-64-0 | 107-44-8 |
| Physical Appearance | Colorless to straw-colored liquid, similar in appearance to motor oil | Water-white to dark yellow | Colorless liquid |
| Odor | Odorless | Odorless | None when pure |
| Viscosity @ 25 oC | 9.96 centistokes | 5.67 centistokes | 1.28 centistokes |
| Solubility (g/100 g Solvent) | Distilled water: 3 @ 25 $^oC$; 7.5 at 15 $^oC$. Readily soluble in organic solvents | Distilled water: miscible below 77 $^oC$. Soluble in most organic solvents. | Miscible with water and readily soluble in all organic solvents |
| Liquid Density(g/cc) | 1.0083 g/mL @ 20 $^oC$ | 1.0312 g/mL @ 25 $^oC$ | 1.09 g/mL @ 25 $^oC$ |
| Vapor Density (Air = 1) | 9.2 | 8.3 | 4.8 |
| Volatility @ 25 oC | 10.5 $mg/m^3$ | 27.3 $mg/m^3$ | $2.2 \times 10^4$ $mg/m^3$ |
| Vapor Pressure @ 25 oC | 0.0007 mm Hg | 0.0021 mm Hg | 2.9 mm Hg |
| Flash Point | 159 $^oC$ | 236 $^oC$ | Does not flash |
| Freezing Point | Below-51 $^oC$; calc. to be –39 $^oC$ | -50 $^oC$ | -56 $^oC$ |
| Boiling Point | 298 $^oC$ | (slightly lower than VX) | 158 $^oC$ |

Source: DA, 1974

#### 2.3.1.1 Vapor Intoxication

The primary signs and symptoms of acute, nerve agent vapor intoxication are constriction of the pupils (miosis), runny nose (rhinorrhea), and increased salivary secretions. In addition, relatively low concentrations of VX vapor produce cutaneous fasciculations (Bramwell *et al.*, 1963). Higher concentrations can cause muscular weakness and tremors, difficulty breathing, convulsions, paralysis, and death (Ward, 1958). Although nerve agents may be absorbed through any body surface, the routes through which absorption is most rapid and complete are the eyes and the respiratory tract. (A more detailed accounting of the signs and symptoms of nerve agent vapor intoxication was given by Grob (1956 a,b) and is reiterated in Table 2.)

#### 2.3.1.2 Local Responses

Local responses result from the action of vapors or aerosols at the site of contact, *e.g*. the eyes, respiratory tract or skin. (Systemic responses occur following systemic absorption at sites distant from the initial point of exposure.) They are caused by inhibition of tissue ChEs at the site and correlate poorly with inhibition of the blood ChEs. Respiratory and ocular effects will precede percutaneous effects—unless there is respiratory and ocular protection. Local responses may be the only effects overtly manifested at low vapor concentrations and will precede systemic effects.

Ocular absorption affects the smooth muscles of the eye, resulting in miosis and spasm of the ciliary body, which makes visual accommodation difficult and painful. Other effects include conjunctival congestion, and eye-associated headache and browache. Miosis is one of the more sensitive indicators of nerve agent exposure and can occur in the absence of inhibition of the blood ChEs. Absorption *via* the respiratory tract affects the smooth muscle and secretory glands of the bronchi, producing tracheobronchial constriction and excessive secretions in the upper and lower airways. The result is watery nasal discharge, tightness of the chest, and wheezing, secondary to the combination of bronchoconstriction and increased bronchial secretion. Percutaneous (skin) absorption produces localized sweating and muscular fasciculation at the site of absorption.

#### 2.3.1.3 Systemic Responses

At relatively higher vapor concentrations nerve agent is absorbed from the respiratory tract and carried throughout the body by the circulatory system. When the agent concentration is sufficiently high, widespread systemic effects may occur in a matter of minutes.

The most prominent systemic effects reported following accidental, sub-lethal, human exposures to VX are gastrointestinal—nausea, vomiting, diarrhea, abdominal discomfort, and pain. Headache, weakness of the eye muscles, and fatigue have also been reported (Bertino *et al.*, 1957).

Severe intoxication is manifested by salivation, respiratory compromise, nausea and vomiting, involuntary defecation and urination, sweating, lacrimation, bradycardia and hypotension, respiratory depression, collapse, convulsions, and death. The proximal cause of death

following acute intoxication from VX (or other anti-AChE compounds) is respiratory failure. The attack upon the respiratory system occurs at several levels: a) tracheobronchial constriction and excessive secretions, b) paralysis of the diaphragm and other respiratory muscles, and c) depression of the respiratory center of the CNS. The predominant site of respiratory failure or embarrassment varies with the species (Koelle, 1994) and the route of exposure and may be local, rather than central.

#### 2.3.1.4 Estimated Effective Dosages

The effects of acute intoxication secondary to VX vapor exposure will be a function of the respiratory minute volume (MV), vapor concentration, and exposure duration. The assumption that the toxic signs and symptoms resulting from a given Ct or total dosage (mg min/$m^3$) will be constant, independent of exposure concentration (mg/$m^3$) and duration (min) (Haber's Law), is invalid for the nerve agents. For the G-agents, exposure to relatively high concentrations, even for a few minutes' duration, will produce much more severe effects than exposure to relatively low concentrations, for long exposure durations. The converse may be true for VX (Crook *et al.*, 1983).

VX vapor intoxication will result from vapor exposure to and absorption from the eyes, respiratory tract, and skin. The ensuing clinical signs and symptoms will be produced by both local and systemic effects. The sequence and intensity of particular signs will depend upon the exposure conditions, especially the concentration of agent and the exposure duration. Human estimates for VX for different endpoints following acute, short duration exposures are given in Table 3. (Human estimates for GB are included for comparison of estimated relative potencies.) A recent review of the available VX data for acute, short exposures to relatively high vapor concentrations (Reutter and Wade, 1994; COT, 1997) indicates that the slopes for lethality and severe non-lethal effects are about 6. Given the potency of VX and normal biological variation, there is likely to be considerable overlap of the different endpoints, and it is not realistic to assign dose-bands**.**

The Army Dispersion Code (D2PC) values for 1% lethality and no deaths are 4 mg min/$m^3$ and 2 mg min/$m^3$, respectively. The authors of the Chemical Stockpile Disposal Program Final Programmatic Environmental Impact Statement (PMCD, 1988), estimated the no-death level for susceptible subpopulations (infants and elderly) to be 20% of the D2PC code value for adult no death (*i.e.* 20% of 2 mg min/$m^3$ or 0.4 mg min/$m^3$).

**Table 2 Signs and Symptoms of Nerve Agent Poisoning**

| Site of Action | Signs and Symptoms |
|---|---|
| | ***Following Local Exposure*** |
| ***Muscarinic*** | |
| Pupils | Miosis, sometimes unequal |
| Ciliary body | Frontal headache; eye pain on focusing; dimness of vision; occasional nausea, vomiting |
| Conjunctivae | Hyperemia |
| Nasal Mucous Membranes | Rhinorrhea; hyperemia |
| Bronchial Tree | Tightness in chest, prolonged wheezing on expiration, cough |
| Sweat Glands | Sweating at site of exposure to liquid |
| ***Nicotinic*** | |
| Striated Muscle | Fasciculations at site of exposure to liquid |
| | ***Following Systemic Absorption*** |
| ***Muscarinic*** | |
| Bronchial Tree | Tightness in chest, prolonged wheezing on expiration, dyspnea, chest pain, increased bronchial secretion, cough, pulmonary edema, cyanosis |
| Gastrointestinal | Anorexia, nausea, vomiting, abdominal cramps, epigastric and substernal tightness with heartburn and eructation, diarrhea, tenesmus, involuntary defecation |
| Sweat Glands | Increased sweating |
| Salivary Glands | Increased salivation |
| Lachrymal Glands | Increased lachrymation |
| Heart | Slight bradycardia |
| Pupils | Miosis, occasionally unequal |
| Ciliary Body | Blurring of vision |
| Bladder | Urinary frequency, involuntary micturition |
| ***Nicotinic*** | |
| Striated Muscle | Easy fatigue, weakness, muscular twitching, fasciculations, cramps, generalized weakness including muscles of respiration |
| Sympathetic Ganglia | Pallor, occasional elevated blood pressure |
| ***Central Nervous System*** | Giddiness, tension, anxiety, jitteriness, restlessness, emotional lability, excessive dreaming, insomnia, nightmares, headache, tremor, apathy, withdrawal with depression, altered frequency spectrum of spontaneous EEG, drowsiness, difficulty in concentrating, slowness of recall, confusion, slurred speech, ataxia, generalized weakness, coma with absence of reflexes, Cheyne-Stokes respiration, convulsions, depression of respiratory and circulatory centers with dyspnea, cyanosis and fall in blood pressure |

Adapted from Grob, 1956a,b

**Table 3 Estimated Effective Dosages (Cts) for VX Vapor Exposure Compared with GB**

| | VX | | GB | |
|---|---|---|---|---|
| **Effect** | **Existing** | **Recommended** | **Existing** | **Recommended** |
| Percutaneous Vapor[a] (mg min/$m^3$) | | | | |
| $LCt_{50}$ | --- | 150 | 15,000 | 10,000 |
| $ECt_{50}$[d] (severe) | --- | 25 | --- | --- |
| $ECt_{50}$[e] (threshold) | --- | 10 | --- | 1,200 |
| Vapor Inhalation[b] (mg min/$m^3$) | | | | |
| $LCt_{50}$ | 30 | 15 | 70 | 35 |
| $ECt_{50}$ (severe) | 25 | 10 | 35 | 25 |
| Ocular or Nasal Vapor[c] (mg min/$m^3$) | | | | |
| $ECt_{50}$[f] (mild) | 0.09 | 0.09 | 2 | 0.5 |

[a] 30-50 minute exposure

[b] 2-10 minute exposures at 15 liters/min

[c] 2-10 minute exposures and are independent of MV

[d] "severe" effects include prostration, collapse, and convulsions

[e] "threshold" effects include slight ChE inhibition

[f] "mild" effects include miosis, rhinorrhea, and tight chest

All estimates are for moderate temperatures of 65-75 $^oF$.

Adapted from Reutter and Wade, 1994 and COT, 1997

### 2.3.2 Central Nervous System (CNS) Effects

Inhibition of brain ChE results in overactivity or blockade of the cholinergic synapses and can lead to abnormal activity in many other neurons. The complex circuitry of the brain provides many opportunities for effects at other sites. The CNS effects, thus, manifest as impairment of motor and cognitive function, changes in electrophysiological parameters, mood or emotions, agitation, confusion, psychosis, delirium, coma, and seizures.

In addition, a number of other neurotransmitters and bioactive substances can be affected directly or indirectly by cholinergic agonists (Glisson *et al.*, 1972). These include the gamma-amino butyric acid (GABA) system, which is important in brain excitability and epileptogenesis (Bowery *et al.*, 1976) and those systems involving peptide transmitters and bioactive peptides (O'Neill, 1981). Whether such effects are brief or long lasting is unknown. However, the circuits are so complex that even a temporary perturbation might lead to reverberations that would persist for a long time. The biological significance of such perturbations is only speculative at this time.

Annau (1992) concluded that the human literature on acute effects of nerve agents on cognition and the persistent nerve agent-induced EEG changes indicated that OP compounds can have long-lasting effects, even after serum ChE has returned to normal levels. Annau cited data on chronic Soman studies in animals (Russell *et al.*, 1986). These studies indicated that behavioral tolerance as able to mask intoxication. Coupling both sets of observations, Annau stated, "…in all species, examined and at all ages, exposure to these compounds can have deleterious and long-lasting, perhaps irreversible consequences."

The human data for the CNS effects of nerve agents, in general, and VX, in particular, are extremely limited. Most of the human data on the neurotoxicity of OP compounds are from occupational exposures to insecticides. The following discussion thus includes data from insecticide exposures in order to illustrate the possible scope of behavioral effects of OP compounds as a class.

#### 2.3.2.1 Organophosphate (OP) Pesticides

Metcalf and Holmes (1969) performed both behavioral and electrophysiological studies on industrial and agricultural workers who had been exposed to OP pesticides. The most obvious signs of intoxication were disturbed memory and difficulty in maintaining alertness and attention. Electroencephalograms (EEGs) showed waveforms suggestive of narcolepsy, perhaps corroborating the inability to maintain alertness. Levin and Rodnitzky (1976) reviewed the effects of OP compounds in humans, in both experimental and industrial settings, and concluded that the most important signs of intoxication were memory deficits, linguistic disturbances, depression, anxiety, and irritability.

Headache, giddiness, paresthesias (numbness or tingling), and ocular symptoms were most commonly observed in workers exposed to Fenthion (O,O-dimethyl-O-(4-methylmercapto-3-methylphenyl)-phosphorothioate). These workers also had significantly reduced serum ChE levels (Misra *et al*., 1985). However, symptoms of intoxication, such as memory loss and impaired cognitive function have been reported to persist, even after serum ChE levels have returned to baseline levels (Coye *et al*., 1986; Savage *et al*., 1988). This suggests that the repeated exposure of human subjects to some OP compounds can have prolonged or chronic effects—even after the usual biochemical indices of exposure, have returned to normal.

There have been reports (Gershon and Shaw, 1961; Dille and Smith, 1964) of psychiatric disturbances following exposure to OP pesticides. However, at least one of these individuals had a previous history of psychiatric problems, and the available data do not show a clear-cut, causal relationship between OP exposure and subsequent mental problems. Stoller *et al.* (1965), in an epidemiological survey, were unable to find any correlation between exposure to OP compounds and the development of psychiatric sequelae, particularly schizophrenia and depressive states.

### 2.3.2.2 Organophosphate (OP) Nerve Agents

The CNS effects of nerve agents do not necessarily equate with those of the OP pesticides. Subacute, sublethal doses of GD do not produce the same effects as diisopropyl fluorophosphate (DFP) on ACh and choline (Ch) concentrations in different regions of the rat brain, and the animals become hyperreactive (Shih *et al.*, 1987). Similarly, in an acute rat study of the effects of VX following intramuscular (IM) injection (10 to 39.8 µg/kg; n= 7/group), Haggerty and Kurz (1985) observed

> "...some of the surviving animals in the 20 and 25.1 µg/kg dose groups showed relatively long-lasting signs of toxicity such as persistent tremoring (seen at 2 days after exposure), seizures, and irritability when handled (both effects seen up to 14 days). The result of this study suggest that VX is a potent nerve agent that causes behavioural changes in the rat that are marked and long lasting."

Credence to the above was provided by a sub-chronic rat study. Subcutaneous injection of VX (0.25, 1.0, or 4 µg/kg) for 120 days produced increased irritability and aggressiveness in the animals in the high dose group, early in the study. Later in the study, the 1.0 µg/kg group showed increased irritability and aggressiveness, and the high dose group exhibited decreased grooming and lethargy. The effects were more pronounced in the males. Taken together, these data do not rule out the possibility of similar effects in humans following nerve agent exposure (Goldman *et al.*, 1988).

There have been several reports of brain pathology in animals surviving relatively high doses of nerve agent, including VX. However, some authors attribute the findings to anoxia (Thornton and Brigden, 1962) or epileptiform activity (McDonough *et al.*, 1995). The occurrence of such findings in the absence of anoxia or seizures has not been demonstrated.

The psychological changes in humans that have been associated with mild intoxication with the nerve agent GB are irritability, absent-mindedness, disturbed sleep, nervousness, inability to concentrate, and fatigability (Ward, 1958). Grob and Harvey (1953, 1958) described the CNS effects of human subjects exposed to repeated oral administration of GB. Signs of muscarinic poisoning, *e.g.* anorexia, nausea, and tightness of the chest, abdominal cramps, vomiting, diarrhea, salivation, and lacrimation, appeared along with CNS effects consisting of tension, anxiety, emotional lability, and insomnia. With exposure that is more prolonged, headache, drowsiness, mental confusion, and slowness of recall were additionally noted. EEG changes consisting of a greater percentage of slow waves and increased amplitude were also observed. Bowers *et al.* (1964) studied the behavioral effects of VX in humans and noted responses very similar to those described above. Subjects had difficulty concentrating and remembering tasks they had to perform and were somewhat irritable. They also had trouble maintaining a train of thought.

Changes in both the human and monkey EEG have been reported to persist for at least one year following exposure to GB (Duffy *et al.*, 1979; Duffy and Burchfiel, 1980). Duffy *et al.* (1979) concluded that the observed EEG abnormalities, coupled with known long-term behavioral effects resulting from OP exposure, indicated that OP exposure might produce long-term

changes in brain function. Although the significance of this has been debated, it has not been shown that it is not an adverse effect, and the USEPA (1995) considers such long-lasting or chronic changes to be adverse effects.

Jager *et al.* (1970) reported electromyographic (EMG) abnormalities in pesticide production workers exposed to OPs and organochlorine (OC) compounds, but not to OC compounds alone. The findings were interpreted to be the result of exposure to the OP compounds and were not associated with ChE inhibition. Roberts (1977) has reported that EMG voltage was significantly depressed in men occupationally exposed to OP pesticides. The voltage depression reflected their pattern of work exposure and was not associated with any clinical signs or symptoms of anti-ChE effects. A recently reported human study by Baker and Sedgwick (1996) indicates that GB vapor-induced EMG changes may be observed for at least two years post-exposure.

The Committee on Toxicology, National Research Council (NRC), was requested by the US Army to study the possible chronic or delayed adverse health effects incurred by those who participated in chemical agent testing at Edgewood Arsenal during 1955-1975. The responses to questions about current health status by subjects exposed to these chemicals suggest that, as a group, these subjects were no different from a control comparison group or from the remainder of the test subjects. If subtle changes had occurred, they were not revealed by the subjects' answers. Post-test admission to Army or VA hospitals for mental disorders did not appear to be significantly increased, either during the years immediately following testing, or later. There was a borderline significant increase in malignant neoplasms among soldiers who had been exposed to anti-ChEs and were admitted to VA hospitals (but not Army hospitals), as compared with those who did not participate in chemical testing. The neoplasms occurred at various sites, and no consistent pattern was seen. National Cancer Institute studies of animal bioassays for carcinogenesis, at maximal tolerated does of ten anti-ChE OP insecticides, indicated that, as a pharmacological class, anti-ChE compounds were unlikely to have induced the malignancies among Edgewood subjects (NRC, 1985).

#### 2.3.2.3 Organophosphate-Induced Delayed Neuropathy (OPIDN) and Intermediate Syndrome

Many OP esters, that may or may not also display anti-ChE properties, produce degeneration of specific regions of the nervous system in humans and several animal species (Johnson, 1975; 1981; Wagner, 1983; Faust and Opresko, 1988). The syndrome first received widespread attention in the 1920s, when some 20,000 cases developed in the southern United States among persons who drank "Jamaica Ginger" that was adulterated with the OP ester TOCP (Smith *et al.*, 1930).

Organophosphate-induced delayed neuropathy (OPIDN) results from direct cellular damage caused by inactivation of a specific enzyme, neurotoxic esterase (NTE) and is not specifically related to inhibition of AChE (Osman *et al.*, 1996). The duration and severity of the disease are functions of the neuropathic potency of the OP compound (not all OPs inhibit NTE) and the exposure history. Severe sensorimotor neuropathy can result from even a single exposure, but does not develop immediately. The typical clinical course consists of an asymptomatic period of 5

to 30 days followed by some initially mild symptoms, such as weakness, tingling, and muscle twitching in the legs. There is subsequent development of flaccid paralysis in the legs and later progression to the hands and thighs. Over time, there is improvement in the lesions induced in the PNS but not those in the CNS (Vasilescu and Florescu, 1980).

The *in vitro* inhibition of hen brain NTE by several nerve agents [GA (Tabun), GB, GD, and VX] was studied by Vranken *et al*. (1982). All of them, except VX, inhibited NTE**.** Similar findings were reported by Gordon *et al*. (1983), who found the ratio of the $I_{50}$s for AChE and NTE to be two to four orders of magnitude, with VX having negligible activity.

In *in vivo* studies, Willems *et al.* (1984) attempted to elicit delayed neuropathy in chickens following injections of GD (up to 1.5 mg/kg) or GA (up to 15 mg/kg)—doses as much as 150 times the $LD_{50}$. Despite aggressive pre-treatment with atropine, physostigmine, diazepam, and HI-6, 50 to 80% of each experimental group died. In the GD-treated animals, delayed neuropathy was observed in only the sole survivor of the highest dose group. In the GA-treated animals delayed neuropathy was observed in 1/2 surviving chickens that had been given two injections of 6 mg/kg.

Somewhat conflicting results were obtained in a repeated inhalation exposure of mice to GB (5 $mg/m^3$, 20 min/day, for 10 days). Beginning 14 days after the first exposure, the animals developed muscular weakness, ataxia, and greater inhibition of NTE than was observed with the OP insecticide mipafox (Husain *et al.*, 1992). Wilson *et al.* (1988) were unable to elicit OPIDN in chickens pretreated with atropine and injected intramuscularly (IM) for 90-100 days with 0.04 mg/kg VX, even although acute effects were observed after each dosing. Goldman *et al.* (1988) similarly investigated the potential for VX to induce OPIDN in the chicken following a single injection of the agent. The animals were pretreated with atropine and 2-PAM and were injected SC with either 10, 100, or 150 µg/kg VX. No indications of OPIDN or other gross damage were observed, and no inhibition of NTE by VX was observed *in vitro*. However, the authors cautioned that the fact that single doses of VX did not cause delayed neurotoxicity does not rule out that it is not neuropathic or myopathic following repeated doses. In reviewing the literature, Munro *et al.* (1994) concluded that VX shows no potential for inducing delayed neuropathy in any species. However, it is noted that there have been no chronic studies of VX in any species, by any route of exposure, and the caution expressed by Goldman *et al.* is quite valid.

An "intermediate syndrome" of neurotoxic effects including paralysis of the respiratory muscles, has been described in several cases of insecticide exposure (Senanayake and Karalliedde, 1987). The onset of symptoms is 24 to 96 hours after poisoning—well after the acute cholinergic crisis has ended and before the expected onset of delayed neuropathy. The muscles involved are different from those that are involved in OPIDN. Nothing is known about the ability of nerve agents to cause this intermediate neurotoxic syndrome; however, it has not been reported following nerve agent exposures. Most frequently, it has occurred following exposure to OP insecticides with long half-lives. It has been hypothesized to result from persistence of the toxic agent, when treated with pharmaceuticals with shorter half-lives.

### 2.3.3 Cardiac Complications

Organophosphorous compounds can produce pronounced cardiovascular effects. There include bradycardia, decreased cardiac output, and hypertension followed by hypotension. OP-induced effects on the electrocardiogram (EKG) include prolongation of the PR interval, A-V conduction defects, T wave and Q-T alterations. Serious ventricular arrythmias, and EKGs indicative of myocardial infarction have also been reported in individuals acutely poisoned with pesticides (McKenzie, 1992).

Serious and often fatal cardiac complications have sometimes occurred with delayed onset following recovery from the acute effects of OP insecticide poisoning (Kiss and Fazekas 1979; Ludomirsky *et al*., 1982; Hirshberg and Lerman 1984; Robineau, 1987). Typically, such complications present as irregularities in the heartbeat (arrythmias). Similar findings have been reported following exposure to several nerve agents, including VX. Salient data are briefly summarized below.

Electrocardiogram abnormalities were reported by Sidell (1973) in one individual following an accidental exposure to GD and in another following an accidental exposure to GB. In the former, the EKG became normal within about 24 hours post-exposure. In the latter, the EKG abnormalities persisted for four weeks and necessitated hospitalization during that time. Four months post-exposure the EKG was "entirely within normal limits". The clinical picture in this individual was atypical of cardiac ischemia.

Transient cardiac arrhythmias were reported in two of the victims exposed to GB in the terrorist attack in Matsumoto. One, a severely poisoned 46-year old woman was admitted to the hospital with atrial fibrillation and cardiopulmonary arrest. A 19-year old woman exhibited intermittent 2:1 AV block for the first three days post-exposure (Suzuki *et al.*, 1997).

Cardiac lesions have also been reported in animals surviving "high" doses of nerve agents (McLeod 1985; Singer *et al*., 1987). However, lesions were reported only in animals surviving convulsions, and all the animals with cardiac lesions had brain lesions. No studies were found indicating whether or not prevention of hypoxia also prevented the cardiac lesions. Electrocardiograms (EKGs) done on dogs following prolonged vapor inhalation exposure to low concentrations of GB revealed elevated "P" waves suggestive of right atrial hypertrophy (Weimer *et al.*, 1979).

In a study of the cardiac effects of VX on anesthetized dogs (6.0 or 3.0 µg/kg SC), Robineau and Guittin (1987) observed decreases in heart rate, arterial and left intraventricular pressures, and the contractility index. Some animals had EKG changes consisting of prolongation of the Q-T interval and arrhythmias (atrioventricular blocks, premature ventricular complexes, and "Torsade de pointe"). The effects on the sinus and atrioventricular nodes were attributed to muscarinic stimulation; however, the more significant effects on ventricular function were attributed to some other, undefined mechanism.

It has been hypothesized that OP-induced cardiac effects (arrhythmias, EKG changes, and lesions) may be secondary to primary effects on the brain following nerve agent and pesticide exposures (McLeod *et al*., 1982; Weidler, 1974). Recently Arsura *et al*. (1987) described complications that can result from the use of anti-ChE medication in patients who already have cardiac problems. A possibility exists, therefore, for preexisting cardiac problems to be exacerbated by nerve agent exposure, although there is no direct evidence for this.

### 2.3.4 Mutagenicity, Carcinogenicity, Teratogenicity and Reproductive Toxicity

Except for an epidemiological follow-up survey of humans exposed to chemical warfare agents (NRC, 1982), no data could be found to assess mutagenicity, carcinogenicity, teratogenicity and reproductive toxicity in humans. Therefore, the potential for such effects of VX will be inferred from animal and *in vitro* studies.

#### 2.3.4.1 Mutagenicity

There are a number of tests designed to determine whether a chemical can damage deoxyribonucleic acid (DNA). Because DNA provides the fundamental code for normal cell function, permanent changes in DNA, or mutations, can result in cell death and permanent changes in cell function. If these mutational events occur in germ cells in the ovaries or testes, the results might be passed on to the next generation as inherited abnormalities. Damage to the DNA of other cells can result in the transformation of a normal cell into a malignant or cancerous cell (carcinogenesis). Damage to the DNA of cells in a developing fetus can result in death or transformation of a cell leading to abnormal development (teratogenesis). For these reasons, the tests for DNA damage by nerve agents are important in assessing the possible human health hazards presented by nerve agents.

Organophosphate compounds that are structurally related to nerve agents have given positive results in certain tests for DNA damage (Malhi and Grover, 1987; Nishio and Uyeki, 1981). However, with other assays and other compounds, there have been negative results or evidence of only weak mutagenicity (Velazquez *et al*., 1986, 1987). It is important that the nerve agents be submitted to a variety of assays before conclusions are drawn as to their ability to damage DNA. Nishio and Uyeki (1981) obtained positive findings in 9/10 OPs using the sister chromatid exchange (SCE) assay, which tests for chromosomal damage, rather than mutations. At least one review of the potential mutagenicity of VX (PMCD, 1988) has indicated that the data are incomplete, and further studies were recommended.

Crook *et al.*, 1983 evaluated VX for mutagenicity using the micronucleus test, the Ames assay, and a *Drosophila* mutation test. The micronucleus test was performed on five female mice, which were exposed to VX vapor at a concentration of 0.000005 $mg/m^3$, for six hours per day, for nine days. It was also performed on two male and two female mice similarly exposed to a concentration of 0.0002 $mg/m^3$. No chromosome damage was detected. The Ames assay was conducted using five tester strains of *Salmonella typhimurium.* The VX doses tested were 1093, 109.3, 1.09, 0.11 and 2.7 x $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, and $10^{-6}$ µg per plate. The mutation testing in *Drosophila melanogaster* produced one mutation (0.005% of the total number) in the offspring

of the flies exposed to 0.004 mg/m$^3$ for 10 days in two separate tests. No mutations were seen in the flies exposed to 0.000005 mg/m$^3$ for 10 days. The conclusions of the authors were that VX was not mutagenic at the concentrations tested.

Goldman *et al.* (1988) tested the mutagenicity of VX in five strains of *Salmonella* (TA98, TA100, TA1535, TA1537, and TA1538). The quantities of VX per plate was 0.0, 0.01. 0.10, 0.50, 2.50, or 10 µg. Effects on revertent numbers were seen only in TA98. The differences were significant, but no dose-response was observed. In addition, the numbers of revertents in the buffer control exceeded those observed in the plates to which VX had been added. The authors concluded that VX was not genotoxic in these *Salmonella* strains. S9 activation did alter the revertent frequencies, but the differences were not considered biologically significant. Tester strain 1535 was completely unresponsive to either VX or two different positive mutagens. It was further concluded that the lack of VX response in the four other strains—with or without mixed-function oxidase enzymes derived from microsomes involved in OP metabolism, indicated that VX does not induce frame shifts or base substitution mutations in *Salmonella*. The potential for VX-induced recombinational effects in *Saccharomyces cerevisiae* was also investigated by Goldman *et al.* (1988). The cells were exposed to 25, 50, or 100 µg/ml, with and without S9 metabolic activation. None of the treatment groups was significantly different from the buffer control. In a mouse lymphoma assay (Goldman *et al.*, 1988), the concentrations of VX that could be employed (as above) did not produce cytotoxicity, which is usually required for the induction of genotoxic effects. The authors concluded that VX does not induce forward mutations at concentrations of 50 µg/ml, or less. Higher concentrations of agent were not tested because the laboratory was not a surety facility. Thus, these findings must be regarded as inconclusive.

#### 2.3.4.2 Carcinogenicity

No experimental data were found on the carcinogenic potential of VX. A study by the NRC (1982, 1985) on the long-term health effects of nerve agents administered to military volunteers found no evidence of carcinogenic potential. McNamara *et al.* (1973) also noted that there has not been increased cancer in personnel working with VX. However, he recommended that appropriate studies should be considered.

#### 2.3.4.3 Teratogenicity and Reproductive Toxicity

A study by the NRC (1982, 1985) on the long-term health effects of nerve agents administered to military volunteers found no evidence of reproductive effects associated with nerve agent exposure. However, Carnes *et al.* (1985) noted the similarity between VX, GB, and several OP pesticides/insecticides that are known to cause fetal and reproductive problems. Although the available data do not indicate that VX has adverse reproductive effects, the data are quite limited and are for non-airborne exposure routes. A brief summary follows.

Goldman *et al.* (1988) investigated the teratogenic, mutagenic and reproductive effects of VX in Sprague-Dawley rats and New Zealand white rabbits. For rats, 4 µg/kg was determined to be the highest dosage that did not produce overt signs of toxicity; however, lethargy

and RBC ChE inhibition were observed. (Pilot studies indicated that higher doses would result in deaths with repeated exposures.) The lowest dosage used was 0.25 µg/kg, which also produced significant RBC ChE inhibition, but no other signs. The dams were injected with VX on days 6 through 15 of gestation and were sacrificed on day 20 of gestation. The data were analyzed for body weights of fetuses, visceral abnormalities, skeletal abnormalities, and miscellaneous effects (*e.g.*, changes in litter size and sex ratio). Although there were some differences among the dose level groups, the only significant finding in the rats was a deviation from the expected 1:1 sex ratio in the low dose group. The observed differences were attributed to random error, rather than dose-effects of VX. The dose groups for the rabbits were identical; however the dams were dosed on days six through 19 of gestation and were sacrificed on day 29. The following data were statistically analyzed: fetal body weights; litter size; fetal deaths such as early resorptions, late resorptions, and dead fetuses; visceral and skeletal abnormalities, and fetal sex. Analyses by Chi-square indicated significant differences among the four dose groups for all parameters except late resorptions. The data were re-analyzed using the nested ANOVA, which takes into account litter effects, and the dose differences disappeared. It was stated that litter effects accounted for 34-45% of the total variation, except for the late resorptions, in which almost all the variation was random. The conclusion was that there was no statistical or clinical evidence that VX affected any of the parameters of the study.

Goldman *et al.* (1988) also performed a modified dominant lethal study in rats. The "modification" was the inclusion of three phases, each consisting of 50 males (10 per dose group) and 200 females (40 per dose group). In Phase I only the males were dosed; in Phase II only the females were dosed; in Phase III both were dosed. VX (0.25, 1.0, or 4.0 µg/kg) was administered by SC injection. Although some significant differences were seen for some parameters, there were no gross or microscopic changes attributable to VX, and it was concluded that VX did not possess dominant, lethal mutagenic properties. Goldman *et al.* (1988) also performed a three-generation reproductive study in rats. The first generation of animals ($F_0$) was dosed, by SC injection, with 0.25, 1.0, or 4.0 µg/kg VX. These animals were bred, producing the $F_1$ generation. The second-generation animals were dosed identically to their parents (same dose groups) and were bred to produce the $F_2$ generation. All but one of the nested analyses of variance for reproductive parameters were highly significant. However, it was not clear, whether the observed effects resulted from the direct action of the agent on the fetus, or if, they resulted from the indirect effect of the agent on the mother. Three effects were observed that had possible dose-response relationships: pituitary cysts, eosinophilic gastritis, and changes in brain and body weight ratios. [See also data below of Van Kampen *et al.* (1970).] (The chosen route of administration, SC injection, is somewhat unconventional. It was employed by Goldman *et al.* (1988) because their laboratory was not a surety facility and they could not administer the material via vapor inhalation or percutaneous liquid exposure.)

Van Kampen *et al.* (1970) studied 79 pregnant sheep accidentally poisoned with VX (Group 1), 38 pregnant ewes that were dosed with an unspecified amount of VX (Group 2), and 88 offspring of these two groups (Group 3). RBC ChE activity was depressed in Group 1 sheep for four months post-exposure and in Group 2 animals for 3.5 months post exposure. Following lambing, Groups 1 and 2 were bred again. Two deformities were found. One was thought to have occurred prior to the poisoning, and the second was in a lamb with a normal twin. It was

concluded that the OP intoxication had little or no effect on the lambs *in utero* at the time of exposure or on the subsequent reproductive capacity of the exposed ewes.

### 2.3.5 Existing Airborne Exposure Limits (AELs) for VX

Existing AELs for nerve agent VX are summarized in 4. They were published by the CDC in DHHS (1988), and by the Army in DA (1990) and DA (1997a,b). No AELs have been derived for any of the other V agents.

The current exposure criteria for VX (as given by DHHS, 1988; DA, 1990; and DA, 1997a,b) are based upon a report by McNamara *et al.* (1973). The latter is a revision of an earlier report (McNamara *et al.*, 1971), which was rescinded. These criteria were subsequently reviewed by Faust and Opresko (1988). A discussion of both documents follows.

**Table 4 Existing Airborne Exposure Limits (AELs) for VX**

| TYPE | EXPOSURE LIMITS ($mg/m^3$) |
|---|---|
| Unmasked Agent Workers (8-hr TWA) A full facepiece, chemical canister, air-purifying protective mask will be on hand for escape. (The M9, M17, and M40 series are acceptable for this purpose. Other masks certified as equivalent may be used.) | 0.00001 |
| General Population:<br>72 hr TWA<br>Ceiling Value | 0.000003<br>0.00001 |
| Source Emission Limit | 0.0003 |
| Masked Personnel in routine operations (8-hr TWA) a. A NIOSH/MSHA-approved, pressure demand full facepiece SCBA or supplied-air respirator with escape cylinder may be used<br>b. Alternatively, a full facepiece, chemical canister, air-purifying protective mask (that is, M9, M17, M40 series mask, or other mask certified as equivalent) is acceptable. | > 0.00001 to $\leq$ 0.02 |
| Personnel Conducting Emergency Operations or operations in unknown but potentially high agent concentrations (8-hr TWA)<br>a. A NIOSH/MSHA-approved, pressure demand full faceplate SCBA or supplied-air respirator suitable for use in high agent concentrations with protective ensemble.<br>b. The best available respiratory protection and personnel ensemble. If protection in (a) above is not available, a full facepiece, chemical canister, air purifying protective mask with hood is acceptable. Currently, only the M9 series protective mask with M11 canister or M40 series mask is acceptable. | > 0.02 |

Adapted from DHHS, 1988; DA, 1990; DA 1997a,b

### 2.3.5.1 Derivation of Existing AELs

The limits developed by McNamara *et al.* (1973) were based upon "human ability (demonstrated or implied) to recover from poisoning by VX". The starting points for the derivation were "median and acute 'no effect' doses" for several endpoints, given in Table 5.

In explaining the concepts behind establishing control limits, McNamara *et al*. (1973) stated that it is a basic principle that a dose, of any chemical substance, having no detectable effect on an organism is acceptable. What constitutes a detectable effect and whether or not some detectable effects are acceptable depends upon how serious they are perceived to be.

It was considered that some effects might be "threshold lowering or "concealed" effects. Such effects had to be accounted for when developing control limits and could be dealt with by using an accumulation model—in the absence of adequate direct data, so that concealed effects would not accumulate and ultimately become manifest.

In developing their model, McNamara *et al*. (1973) recognized that recovery could be a function of one or more processes. In the case of GB, McNamara and Leitnaker (1971) reported that recovery of RBC-ChE activity paralleled the replacement of senile RBCs and correlated poorly with clinical recovery from toxic signs and symptoms. However, VX was somewhat different in that recovery of ChE activity was more rapid and was thought to reflect some spontaneous dephosphorylation of the enzyme, in addition to synthesis of new enzyme.

**Table 5 Median and Acute No Effects Dose Estimates for VX**

| Effect | Effective Dose | | No Effects Dose | |
|---|---|---|---|---|
| | $ED_{50}$ (µg/kg) | $ECt_{50}$ (mg min/$m^3$*) | $ED_{50}$ (µg/kg) | $ECt_{50}$ (mg min/$m^3$*) |
| Death | 7.5 | 35 | 0.94 | 4.4 |
| Tremors (neuromuscular effect) | -- | -- | 0.34 | 1.6 |
| 50% RBC-ChE inhibition | 1.0 | 4.7 | -- | -- |
| No RBC-ChE inhibition | -- | -- | 0.1 | 0.47 |
| Miosis | -- | 0.09** | -- | 0.02** |

*Based on a breathing rate of 0.2145 liters $min^{-1}$ $kg^{-1}$ (15 liters/minute for a 70-kg man).

**Direct effect on eye. Near-lethal systemic doses are required to produce miosis.

Adapted from McNamara *et al.*, 1973

[Note that many of the above estimates are not supported by the available data; see discussion below.]

McNamara *et al*. (1973) thus stated, "A simple conceptual model of recovery resulting from a single acute dose, when one or more recovery mechanisms are operating independently, was presented in an earlier paper (McNamara and Leitnaker, 1971). In the general case of

the model, recovery from a single acute dose is expressed as the sum of n (2 or more) component exponential functions, *i.e.*,

$$D = D_0 e^{\lambda t} \quad (1)$$

$$E = D_d / \lambda \, (1 - e^{-\lambda t}) \quad (2)$$

$$E = D_d / \lambda, \quad (t \to \infty) \quad (3)$$

where:

| | |
|---|---|
| D | = cumulative effect |
| $D_0$ | = effect present at time, t = 0 |
| $D_d$ | = dosing per unit of time |
| $\lambda$ | = constant with reciprocal time units |
| E | = the acute dose to produce the effect." |

This model was derived from GB data for the development of control limits for GB. It is designed to predict accumulation of effective dose, but not accumulation of effect. It was assumed that the effect of an accumulated dose is equivalent to the same dose delivered acutely. The model, thus, predicted that the accumulated effect would be the effect produced from a dose ~ 18 times the daily dose. It did not predict that the accumulated effect would be 18 times the effect of the daily dose, assuming non-linearity between dose and effect.

The model was based upon recovery of plasma ChE. In humans, plasma ChE is comprised of BuChE. This is not constant across species. In other species, plasma ChE may consist of BuChE, AChE, or various mixtures of AChE and BuChE. It is noted that the effects produced by nerve agents are generally attributed to the inhibition of AChE, and that VX preferentially inhibits AChE.

The justification for using the model for VX was "the pharmacological similarity" between GB and VX and "the adequate fit of available VX data to the model" (Figure 1). This figure was based upon an n of one, from the study of Kimura *et al.* (1960) (six other individuals were included in the study, but they did not receive this dosage). The parameters from the figure were considered consistent with those of the six other individuals in the Kimura *et al.* (1960) report (Figure 2). Assuming that the recovery of plasma ChE activity adequately reflects recovery of toxicity, and using the parameters from Figures 1 and 2 and equation (3), the following was derived

$$D_d = \lambda \times E \quad (4)$$

This equation was assumed to be applicable to both direct effects on the eye and systemic effects through the respiratory tract following vapor inhalation of VX. Based upon the data of Cresthull *et al.* (1963) (Figure 2, also see below), McNamara *et al*. (1973) stated that percutaneous absorption of VX vapors would be negligible. It was further stated that a Ct of at least 100 mg min/$m^3$ would be required to reduce whole blood ChE by 50%, as compared with 4.7 mg min/$m^3$ by inhalation. [This is assumption is not supported by the Bramwell *et al.* (1963) data; see below.]

Based upon the above calculations, it was concluded: 1) Systemically, VX is twice as toxic as GB. 2) VX is 25 times more potent than GB in producing miosis. 3) Recovery from the effects of VX is four times faster than for GB. Effectively, therefore, an allowable concentration to VX was considered to be 4/25 or 0.16 that for GB. The proposed figures were 1/10$^{th}$ those for GB. They are as follows.

McNamara *et al*. (1973) defined "healthy workers" as healthy individuals, medically examined and cleared to work in areas where VX is manufactured, stored, transported, *etc.*

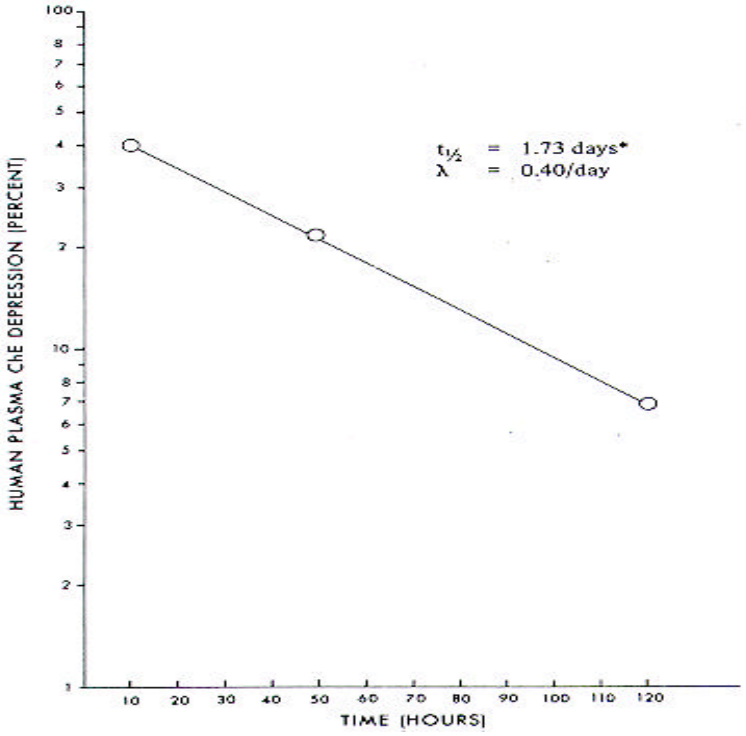



**Figure 1. Rate of Recovery of Plasma ChE Activity in Humans Following IV Administration of VX**

From McNamara *et al.*, 1973

"The effect of VX that appears at the lowest Ct is miosis... Since the 'no effect' dose (miosis) is 0.02 mg min/$m^3$ (Table 6), the [*above*] model predicts that about 0.4 of this dose, *i.e.* 0.008 mg min/$m^3$, could be tolerated daily without the development of pupillary constriction. This dose equates the exposure to an *average* concentration of 0.000017 mg/$m^3$, 8 hours a day, every day, indefinitely. We therefore propose that healthy adult workers, without any protective devices, should not be exposed to an average concentration (averaged over the preceding 5 days) exceeding 0.00001 mg/$m^3$ of VX vapor. **CLWP-Ind/5 days = 0.00001 mg/$m^3$**. [*CLWP-Ind = control limit for the workplace, for an indefinite period.*]

**Table 6 ChE Inhibition Following Inhalation Exposure of Human Subjects to VX Vapor**

| Trial | Subject | Exposure Conditions | | | Max ChE Inhibition (% depression) |
|---|---|---|---|---|---|
| | | Time (min) | Conc. (mg/$m^3$) | Ct (mg min/$m^3$) | |
| R1 | SHE | 3 | 0.2 | 0.6 | 20 |
| R2 | BIS | 3 | 0.35 | 0.9 | 18 |
| R3 | LAD | 3 | 0.31 | 0.9 | 22 |
| R4 | BUR | 3 | 0.37 | 1.1 | 17 |
| R5 | BRA | 3 | 0.4 | 1.2 | 14 |
| R6 | HOP | 3 | 0.48 | 1.4 | 10 |
| R7 | CRO | 3 | 0.57 | 1.7 | 12 |
| R8 | SHE | 1.5 | 1.6 | 2.4 | 26 |
| R9 | BRA | 1.5 | 1.73 | 2.6 | 25 |
| R10 | BUR | 1.5 | 1.73 | 2.6 | 21 |
| R11 | BIS | 1.5 | 1.93 | 2.8 | 28 |
| R12 | LAD | 1.5 | 2.0 | 3.0 | 41 |
| R13 | HOP | 1.5 | 2.07 | 3.1 | 18 |
| R14 | HOL | 1.5 | 2.07 | 3.1 | 28 |
| R15 | CRO | 1.5 | 2.4 | 3.6 | 20 |
| R16 | CRO | 6 | 0.8 | 4.8 | 44 |
| R17 | LAD | 7 | 0.79 | 5.5 | 70 |
| R18 | SHE | 6 | 1.02 | 6.1 | 47 |
| R19 | BUR | 6 | 1.06 | 6.4 | 46 |

Adapted from Bramwell *et al*., 1963

"Only 8 hours a day can be used in averaging, and exposure cannot exceed 8 hours each day. Nonexposure days can be used in the 5-day averaging whether worked or not. However, the average concentration for *any one* 8-hour period should not exceed 0.00002 mg/$m^3$. **CLWP-8hr = 0.00002 mg/$m^3$**.

"Three or more such 8-hour days should not occur in 5 consecutive days, but two could occur if exposure for the other 3 days were adequately curtailed.

"It is further proposed that the average exposure concentration in *any 1-hour period* should not exceed 0.00005 mg/$m^3$. **CLWP-1hr = 0.00005 mg/$m^3$**. Not more than 3 such hours in any single day should be allowed unless exposure for the

remaining 5 hours is negligible; *i.e.*, less than the general population indefinite exposure limit, which will be given below.

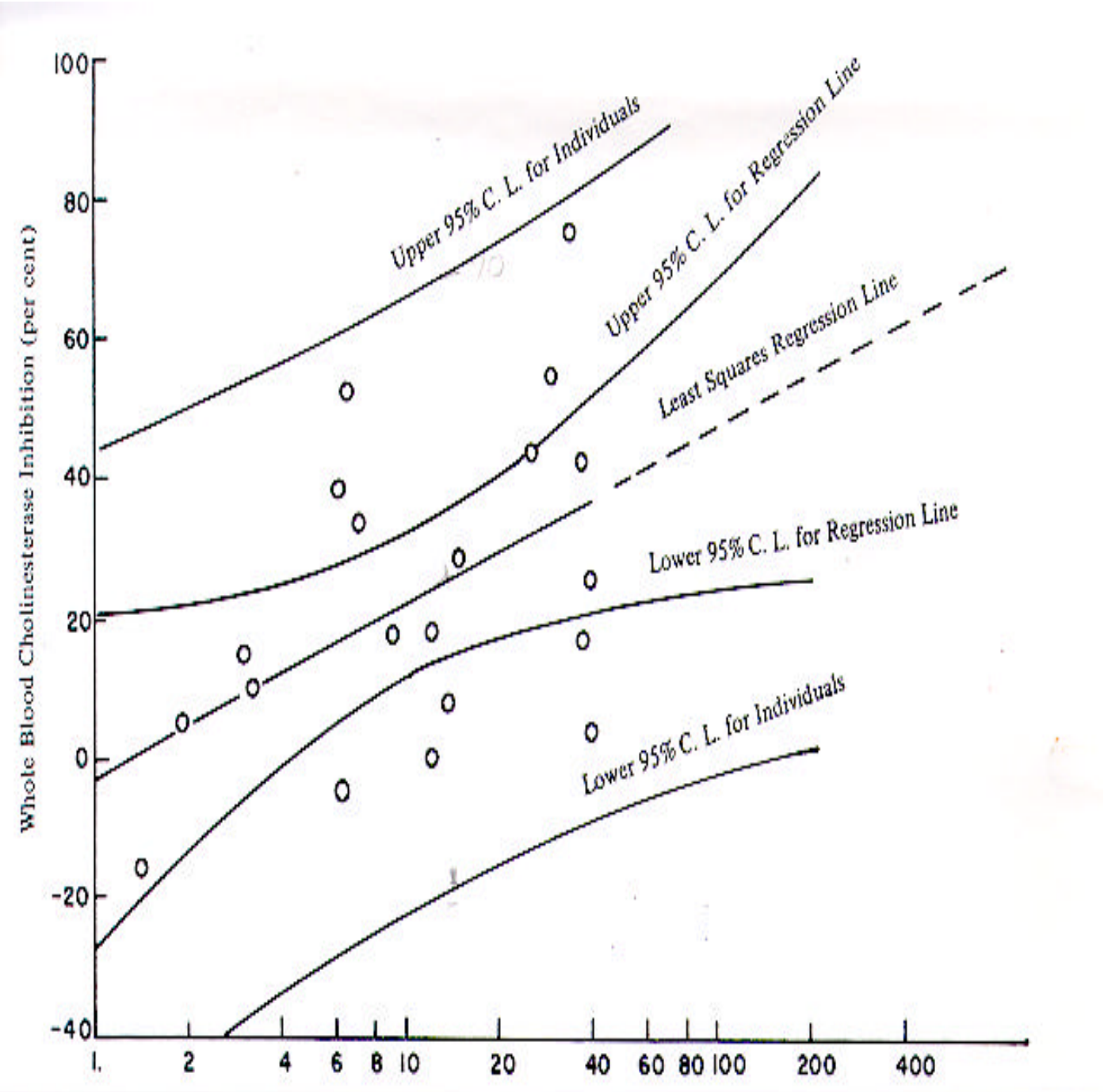
"Since the threshold for pupillary constriction (which is a direct local effect on the eyes) is so far below any other detectable effect of VX, and since the eyes of workers might be easily protected against VX vapor by occlusive goggles, it might be helpful and acceptable to propose control limits for workers whose eyes are so protected. Such concentrations would be based on no detectable depression of RBC or plasma ChE."

Based upon calculations of the predicted dose effect accumulation for RBC ChE depression, McNamara *et al*. (1973) stated the following.

"Since the no effect daily dose for ChE depression is the same as the no effect daily dose for miosis, special control limits for workers with eye protection are not warranted until more data are available." [This assumption is not supported by the data of Crook *et al.* (1983).]

"In establishing environmental concentration limits for GB, a safety factor of 30 (10 for population variability X 3 for 8 hours versus 24-hour exposure) was applied to the values for workers to protect the general population. The same is done here for VX. The repeated 8-hour "no miosis" concentration for VX in unprotected workers (0.00001 $mg/m^3$) divided by the safety factor (30) yields a repeated 24-hour 'no miosis' concentration for the general population of 0.000,000,3 $mg/m^3$. This concentration is proposed as the control limit of VX for the general population for an indefinite time with an averaging period of 72 hours. **CLGP-Ind/72 hr - 0.000,000,3 $mg/m^3$**.

"It is also proposed that the control limit for a 1-hour exposure be 0.000,005 $mg/m^3$. **CLGP-1 hr - 0.000,005 $mg/m^3$**."




From McNamara *et al.*, 1973

**Figure 2 Scatter Diagram of Individual ChE Inhibitions in Man After Percutaneous Arm Exposures to VX Vapor**

**Table 7 Mortality in Several Species Following Sub-Acute Exposures to Low Concentrations of VX Vapor**

| VX Concentration ($mg/m^3$) | Days Exposed | Mouse | | Guinea Pig | | Rat | | Rabbit | |
|---|---|---|---|---|---|---|---|---|---|
| | | # Dead | Sex | # Dead | Sex | # Dead | Sex | # Dead | Sex |
| 0.000005 | 5 | 0/26 | M | 0/25 | M | 0/20 | M | 0/4 | M |
| | | 0/20 | F | 0/25 | F | 0/20 | F | 0/2 | F |
| | 10 | 0/26 | M | 0/25 | M | 0/20 | M | 0/4 | M |
| | | 0/20 | F | 0/25 | F | 0/20 | F | 0/2 | F |
| | | *0/46* | *Tot.* | *0/50* | *Tot.* | *0/40* | *Tot.* | *0/6* | *Tot.* |
| 0.00006 | 5 | 0/10 | M | 0/10 | M | 0/10 | M | 0/8 | M |
| | | 0/10 | F | 0/10 | F | 0/10 | F | 0/8 | F |
| | 10 | 0/5 | M | 0/5 | M | 0/5 | M | 0/4 | M |
| | | 0/5 | F | 0/5 | F | 0/5 | F | 0/4 | F |
| | | *0/10* | *Tot.* | *0/10* | *Tot.* | *0/10* | *Tot.* | *0/8** | *Tot.* |
| 0.0002 | 5 | 0/22 | M | 0/22 | M | 0/22 | M | 0/8 | M |
| | | 0/22 | F | 0/22 | F | 0/22 | F | 0/8 | F |
| | 10 | 0/11 | M | 0/11 | M | 0/11 | M | 0/4 | M |
| | | 0/11 | F | 0/11 | F | 0/11 | F | 0/4 | F |
| | | *0/22* | *Tot.* | *0/22* | *Tot.* | *0/22* | *Tot.* | *0/8* | *Tot.* |
| 0.004 | 5 | 23/23 | M | 2/37 | M | 9/40 | M | 0/8 | M |
| | | 23/23 | F | 1/37 | F | 3/40 | F | 0/8 | F |
| | 10 | - | M | 2/37 | M | 17/40 | M | 0/4 | M |
| | | - | F | 2/37 | F | 11/40 | F | 0/4 | F |
| | | *46/46* | *Tot.* | *4/74* | *Tot.* | *28/80* | *Tot.* | *0/8* | *Tot.* |

*Data given as presented by Crook *et al.*; one non-agent-related death occurred after 5th exposure.

Adapted from Crook *et al.*, 1983

### 2.3.5.2 Discussion of Existing Exposure Standards for VX

Most of the "Doses of VX Estimated to Cause Effects in Humans", as given by McNamara *et al.* (1973) (Table 7, above) were not based on data for inhalation exposures to VX. In fact, some were not even based on data for VX. Those that were based on VX data, were calculated from data for routes of exposure other than inhalation. The others were referenced to existing estimated doses for GB, many of which were derived from data for routes of exposure other than inhalation**.** It was stated that this was done because "There are no data on the effects of in-

haled VX in man". This is somewhat problematic because the initial signs and symptoms characteristic of a whole-body vapor exposure to nerve agent are local effects in both the eyes and respiratory tract. This point was noted by Faust and Opresko in their 1988 report. Specific comments on each estimate follow.

The given "**$LCt_{50}$**" for VX was adopted by the CWL (Chemical Warfare Laboratory) Committee on Human Estimates in 1960. It was based upon the existing $LCt_{50}$ for GB, which was calculated from IV data, in order to compensate for differences in MV among various animal species. Since the available data for VX indicated that it was twice as potent as GB by the IV route, the VX $LCt_{50}$ was estimated to be one-half that for GB. However, a recent review of the available data and existing human toxicity estimates for selected chemical agents (Reutter and Wade, 1994; COT, 1997) indicates the following: 1) The available inhalation (IH) data for GB do not support the existing $LCt_{50}$. 2) The estimate for GB appears to have been derived for offensive purposes—it was intended to kill at least 50% of the least sensitive men on the battlefield, and thus, underestimates the effective dosage in the average human male.

The "**no deaths dose**" for VX was also based upon that for GB. The latter was calculated by transposing assorted GB IH data to 2-minute exposures with a 15 L MV (Solomon *et al.*, 1970). This treatment of the data was dubious for a number of reasons: 1) The actual exposure durations ranged from several seconds to many minutes, and Haber's Law is invalid for these agents (Mioduszewski *et al.*, 1998). 2) Independent of duration, the exposure paradigms were markedly different. Some were oral inhalation, without ocular exposure. Others were nasal inhalation without ocular exposure. Still others were nasal inhalation with ocular exposure. The calculation of the "no deaths dose" was made by comparing estimated $LCt_{01}$ values for GB and VX, based upon probit slopes of 7 for both agents. VX was observed to be 2.3 times more potent than GB. However, the probit slope for GB is at least 12 and that for VX is about 6; thus, at the $LCt_{01}$ VX is approximately 6 times more potent than GB.

The "**no tremors dose**" for VX was similarly calculated from estimated GB doses, based upon the assumption that VX was 2.3 times more toxic than GB in animals, when the probit slopes are compared for low doses. It is further noted that there is a paucity of low dose data for both GB and VX and the shape of the dose-response curve at the low end is unknown.

The "**50% RBC ChE inhibition dose**" for VX was based upon IV, not IH, data in humans. These data are limited to about seven subjects.

The "**no effects' dose for RBC ChE inhibition**" was based upon IV data in one individual, and the individual received the agent in two divided doses given 3.5 hours apart. Although the individual's RBC ChE was within normal limits, he experienced a 2-fold increase in airway resistance, a 25% to 30% decrease in respiratory rate, a 15% decrease in pulse rate, frontal and retrobulbar headaches, an increase in MV from 15 to 32 L, a feeling of sweatiness and lightheadedness, and abdominal cramps. He was also observed to be irritable and to talk less clearly. In short, although there may have been no measurable RBC ChE inhibition, it was not a "no effects" dose.

The **$ECt_{50}$ for miosis** was predicated upon rabbit data for VX and the assumption that man is twice as sensitive as the rabbit. These data have not been found.

The **no miosis** dose was derived from a "no effects" dose of 0.5 mg min/$m^3$ for GB, based upon the assumption that VX is 25 times more potent than GB in producing miosis. (The data on which this assumption was based have not been found). This "no effects" dose for GB was extrapolated from one set of data (McNamara and Leitnaker, 1971) and is not supported by the larger body of available human data (Reutter and Wade, 1994; COT, 1997).

### 2.3.5.3 Other Reviews of the Existing Exposure Standard for VX

In 1988, Faust and Opresko reviewed the general toxicology of VX, reevaluated the current exposure standards from the perspective of the most recent toxicological data, and recalculated an occupational standard. They used both human and animal data and correlated VX dose to the inhibition of RBC ChE following IV or SC administration.

They did not reference the VX inhalation studies of Bramwell *et al.* (1963) or Crook *et al.* (1983). They did present a careful review of McNamara *et al.* (1973) and discussed correlation and lack thereof between ChE inhibition and clinical effects. It was stated, "For the chemical agent VX, there is substantial evidence that miosis and the inhibition of blood AChE are the most sensitive measurable physiological effects." However, it was further stated, "In occupational settings direct contact of VX with the eyes of workers would be avoided by the use of eye protectors such as goggles, therefore, the inhibition of blood-AChE would be the most appropriate parameter to use for limiting exposures to VX." The occupational exposure standards were thus, calculated using both human and animal data RBC-ChE inhibition data with the assumption that there would be no ocular vapor exposure.

For the calculations based upon human data, Faust and Opresko (1988) used the RBC-ChE inhibition curve from the IV data of Sidell and Groff (1974). Based upon the existing guidelines for worker exposure to ChE inhibitors, a LOAEL was considered to be 30% inhibition of RBC ChE, and 15% inhibition was considered to be a NOAEL. Using a generalized formula for human parenteral data (Opresko, 1988), the following was presented:

$$\mathbf{C_A = (NOAEL_{IV})/[RESP_{OCCUP} \times EXP_{OCCUP} \times F_P] \times bw_H \times (1/UF)}$$

where:

| | |
|---|---|
| $C_A$ | = concentration in workplace air |
| $NOAEL_{IV}$ | = no-observed adverse effect level (IV) = 0.72 µg/kg |
| $RESP_{OCCUP}$ | = 43 L/min (occupational breathing volume) |
| $EXP_{OCCUP}$ | = 480 min (occupational exposure period) |
| Fp | = 0.8 (pulmonary availability factor, a standardized pulmonary adjustment factor to account for the presence of dead air spaces in the respiratory tract.) |
| $bw_h$ | = 70 kg (standard human body weight) |
| UF | = uncertainty factors |

uncertainty factors = UF1 x UF2

UF1 = 10 (to adjust for potential individual variability in sensitivity to VX)
UF2 = 18 (to adjust for possible cumulative effects at low exposure levels, based on the model of McNamara *et al.* (1973)

then:

$$\mathbf{C_A = (0.72\ \mu g/kg)/[\ 43\ L/min\ x\ 480\ min\ x\ 0.8]\ x\ 70\ kg\ x\ (1/10\ x\ 18)}$$

$$\mathbf{C_A = 0.000017\ \mu g/L}$$

$$\mathbf{C_A = 0.000017\ mg/m^3.}$$

For calculations based upon animal data, Faust and Opresko (1988) used the data of Goldman *et al.* (1987). In this study, rats were injected SC, either acutely or sub-chronically (five days per week for up to 90 days). Extrapolation of the data indicated that the chronic dose producing 15% RBC ChE inhibition after 30 days would be equivalent to about 0.015 µg/kg. This value was used as a NOAEL, and an occupational exposure standard was calculated from the following formula (Opresko, 1988):

$$\mathbf{C_A = (NOAEL_{SC})/[RESP_{OCCUP}\ x\ EXP_{OCCUP}\ x\ F_P]\ x\ bw_H\ x\ (1/[70/\ bw_A]^{1/3}\ (1/UF)}$$

where:

$C_A$ = concentration in workplace air
$NOAEL_{sc}$ = no-observed adverse effect level = 0.015 µg/kg
$RESP_{OCCUP}$ = 43 L/min (breathing volume)
$EXP_{OCCUP}$ = 480 min (exposure period)
$F_P$ = 0.8 (pulmonary availability factor)
$bw_H$ = 70 kg (standard human body weight)
$bw_A$ = 0.4 kg (standard animal body weight)
$1/[70/bw_A]^{1/3}$ = 0.179 (interspecies adjustment factor for body size differences
UF = uncertainty factor = 10 for intraspecies variability

then:

$$\mathbf{C_A = (0.015\ \mu g/kg)/[43\ L/min\ x\ 480\ min\ x\ 0.8]\ x\ 70\ kg\ x\ 0.179\ x\ (1/10)}$$

$$\mathbf{C_A = 0.0000011\ \mu g/L}$$

$$\mathbf{C_A = 0.0000011\ mg/m^3}$$

Faust and Opresko (1988) concluded: 1) The existing exposure standards are valid. 2) Rats are more sensitive to VX than humans.

However, they were assuming ocular protection, which McNamara *et al.* (1973) were not, so this does not substantiate the existing exposure standards. In addition, given that the rat and human exposure routes were different, the conclusion that rats are more sensitive than

humans is not substantiated. In the absence of data to the contrary, it must be assumed that humans are the species most sensitive to VX.

## 3. FINDINGS/DISCUSSION

Many of the data currently available for consideration were previously classified, are from foreign sources, and/or are from studies that were done *after* the existing criteria for VX were proposed. There are three salient points to consider with regard to the existing criteria for VX and review of the available data for VX. 1) The existing criteria are based upon inhibition of ChE, or recovery from inhibition of ChE. VX preferentially inhibits AChE, and the enzyme used as the basis for the above enzyme-recovery model was BuChE. Moreover, adverse effects can occur in the absence of any blood enzyme inhibition, particularly following airborne exposure. 2) Several critical assumptions were based upon estimated effective dosages for VX that were *calculated* from estimated dosages for GB—the critical VX doses were not derived from VX data. Some of these estimates are not supported by the available human data for VX. 3) The VX data that were used were not for vapor inhalation exposures. The clinical signs and symptoms occurring following VX exposure (or exposure to any such agent) depend markedly upon the route of exposure, and following airborne exposure the critical effect will likely occur in the absence of blood ChE inhibition.

### 3.1 V-Agent Exposure Data

The V-agent exposure data presented herein either have been previously used in the development of VX criteria or are pertinent to the development of such criteria. They include both animal and human studies. None of the data are for chronic exposures; there have been no chronic toxicity studies on VX, in any species, by any route of exposure.

#### 3.1.1 Human Exposures

The controlled human exposure data for V agents are very limited, particularly for vapor inhalation. The following discussion includes several reports of accidental human V-agent exposures, because it is important to comprehend the likely course of clinical events following exposure.

##### 3.1.1.1 Accidental Exposures

Freeman *et al.* (1956) wrote one of the first reports of any human intoxication with V-agent. It discusses five accidental human exposures that occurred at the Army Chemical Center (now the Edgewood Area of Aberdeen Proving Ground). The report implies that no human testing had been done at that time, because the V agents were so toxic in animal studies there was reluctance to test them in humans. 1) The first exposure occurred in August of 1954. An individual working with VG and wearing only protective glasses was splashed on the hands and face when a container exploded. The clinical manifestations of intoxication were mild miosis and eye pain. There was apparently significant ChE inhibition, but he was recovering from a previous ex-

posure to a ChE inhibitor, and it is difficult to determine what percentage of the reduction in ChE activity was due to the VG exposure. 2) Another individual was exposed while weighing a sample of VG. The initial symptoms were dim vision and then dizziness. These were followed by nausea and vomiting, abdominal cramps, fluttering of one eyelid, pallor and perspiration, weakness and fatigue, and photophobia. Based on recovery of activity over the ensuing five weeks, RBC ChE activity appeared to have been inhibited by at least 85%. 3) A third individual was exposed to VE, most likely by rubbing his eyes with contaminated hands. Bilateral miosis was obvious the next day. ChE determinations two days after exposure indicated some inhibition of plasma ChE ($\geq$ 40% based on recovery of activity), but no inhibition of RBC ChE. (VX preferentially inhibits AChE, but this is not necessarily true of the other V agents.) At that time, he still had some dimness of vision in the left eye and occasional fasciculations of the periorbital muscles. The following day he experienced abdominal discomfort and loose bowel movements. 4) A fourth individual was a woman who was also exposed to VE. She had previously worked with (and been exposed to GB) and had been working with V agents for about four weeks. The exposure occurred while she was pipetting the material in a hood. Her head was outside the hood, and she was wearing a gas mask, rubber gloves, and a lab coat. During the procedure, she extended her arms within the hood, and the sleeves of her lab coat came out of her gloves, exposing her forearms. That night she had a headache, but she did not have any pupillary effects or visual disturbances. The following day she experienced unusual sweating of the forearms, wrists, and face and a sensation of "pressure on the chest". Her most disturbing symptoms were fatigue and "heaviness of the limbs", which persisted for a week. It was also noted that the day after exposure she abruptly stopped menstruating. Her RBC ChE activity was about 80% of normal. 5) The fifth exposure was an individual who was experiencing weakness of the eyelids and had virtually complete inhibition of ChE activity. The case was reported in detail by Bertino *et al.* (1957), and it is discussed further below.

Grob and Johns (1956) described the clinical effects observed in two individuals accidentally exposed to VG. 1) The first individual had worked with the material for two weeks and had been handling it for an hour on the day of exposure. The route of exposure is not known, but the operations in which he was involved afforded the possibility of percutaneous, oral, and/or inhalation exposure. His symptoms were, in order of appearance: a) slight nausea; b) slight dimness of vision (one eye worse than the other), with miosis and fluttering of the eyelid; c) moderate cold sweat, slight gray pallor, and anxiety; d) significant ChE inhibition; e) vomiting; f) photophobia (one eye worse than the other); and g) increased bowel sounds and activity. Clinical chemistries—other than ChE activity, were normal, and an EEG the following day was normal. The onset time of the symptoms was longer than expected after exposure to a comparable dose of GB. 2) The second individual probably touched his eyes after handling VG. The presenting symptoms were sudden, sharp pain behind the left eye and in the frontal and parietal regions; dim vision in the left eye, with pupillary constriction and twitching of the periorbital muscles. The next day he had abdominal cramps and loose bowel movements, and felt "tense and 'nervous'". Vision in his left eye was still dim, and the pupil was markedly constricted. His plasma and RBC-ChE activities were not significantly depressed.

Bertino *et al.* (1957) presented sixteen cases of V-agent exposures, thirteen of which were "previously unreported". The individuals categorized as follows: Group A: no symptoms; lowered ChE values discovered on routine check; Group B: low ChE discovered on

routine check, symptoms apparent on questioning; Group C: symptoms causing the individuals to seek attention.

Four individuals comprised Group A. All had been working with V-agents.Only one individual realized he had been exposed, and it is known that the route of exposure was percutaneous (liquid VX and analogous compounds in ethanol). One individual was wearing gloves and a protective mask while pipetting VX and VM in a hood. Another was wearing gloves and a laboratory coat while working with EA 1533 in a hood. Exposure circumstances were not given for the fourth person, who was working with VX and VS. Although, with the exception of one person, the route of exposure cannot be documented for these individuals, it would appear that at least 3/4 did not have an inhalation exposure. The RBC ChE activities of the group ranged from 30% to 71% of baseline.

Two individuals were in Group B. 1) One person had been wearing gloves,apron and boots while working with EA 1671. That evening he had cramps and diarrhea, anxiety, insomnia, and mood changes . The next day he had excess sweating on the backs of his hands and around his wrists. His RBC ChE activity was 11% of normal. 2) The other individual was apparently exposed by "unavoidable" volatilization of EA 1671 crystals, which he was melting. He had no symptoms the first day of exposure. The following day, while continuing the operation, he noted weakness of the eyelids in that it was difficult for him to open and close them. He also developed diarrhea and abdominal distress. The following day, when he had a routine ChE test, it was discovered that his RBC ChE activity was 15% of normal.

Ten individuals formed Group C [two of them were reported by Grob and Johns (1956) and were described above]. 1) One was a chemist who had been involved in the synthesis of V agents for several months. Upon questioning he admitted to weakness of the eyelids and having difficulty opening them after they had been closed for some time. His RBC ChE activity was nearly zero. 2) Another person had been working with two agents, VE and VM. One of his co-workers noticed that his eyes were inflamed, and he had pin-point pupils. His RBC ChE activity was about 15% of normal. 3) Another individual had no obvious circumstances of exposure. "After leaving work on a Friday, he had a headache across his forehead Saturday evening, and Sunday had a continuous 'pain in the middle of his stomach,' persisting until Monday." When he sought medical aid, it was discovered that his RBC ChE activity was 24% of normal. 4) Another person was working with some equipment that was probably contaminated with EA 1533. After brushing his left eye with his hand, he noticed twitching of the eyelids, blurred vision, and miosis of the left eye. His RBC ChE activity was 61% of normal. 5) One person splashed a solution of a derivative of VX in an ether-ethanol solution into his eye. Despite immediate decontamination, the next morning, he had unilateral miosis, and his RBC ChE activity was about 50% of normal. 6) The only woman in the group had been using different methods to hydrolyze VX. Her initial symptoms/signs were abdominal cramps, diarrhea, and malaise for a week. About that time, she noticed a smell about her laboratory apparatus, and she flushed the system with water. On her way home, she had to stop her car because of abdominal pain, nausea, and vomiting. The pain persisted and was accompanied by diarrhea, anxiety, insomnia, mood changes, fatigue, and blurred vision. Her RBC ChE activity was 83% of normal. 7) Another person was apparently exposed, at the end of the day, while washing glassware that had contained V-agent derivatives, mainly VX, and had been decontaminated with hypochlorite. Ten minutes later he developed rhi-

norrhea. He drove home. The rhinorrhea persisted. He was unable to concentrate to read, was anorexic, and began to develop shortness of breath and tightness and pain in his chest, shoulders, and arms. He felt worse upon lying down and had difficulty sleeping. When he sought treatment the next day, he was noted to have bilateral miosis. No values were given for his baseline RBC ChE activity, but inhibition appeared to be negligible—his ChE activity one month post-exposure was not significantly different from that at the time of exposure. 8) The last individual was involved in injecting liquid VX into an aerosol generator. He wore a mask, glove and boots and decontaminated his gloves after each injection. About two hours after completing the procedure he noticed that things seemed dark, and he had difficulty reading the newspaper. He was also experiencing mild rhinorrhea, headache, and fatigue. Physical examination additionally revealed bilateral miosis and mild conjunctivitis. Some generalized sweating was later experienced. His RBC ChE activity was about 57% of normal.

It is interesting to note that, although the V-agents are not considered particularly volatile, nearly half of the above exposures would appear to have resulted from airborne agent, and percutaneous vapor absorption was significant.

#### 3.1.1.2 Controlled Exposures

Koon *et al*. (1959) studied sixteen volunteers who participated in an odor detection study of stabilized and unstabilized VX. The agent was inhaled through an osmoscope attached to a gassing chamber containing a VX vapor concentration of 3.34 $mg/m^3$. The osmoscope permitted dilutions of the agent vapor with room air to yield concentrations down to $1/64^{th}$ that in the chamber. Each subject participated in several "sniff" tests. The estimated total dosages to which they were exposed ranged from 0.01 to 0.13 µg/kg. No significant changes in ChE activity were demonstrated. Three subjects reported headaches the evening of the last test, and three other subjects reported slight chest tightness, dryness of the mouth and nasal irritation for 30 minutes following the test. Interestingly, there was no agreement as to the description of the odor. The median detectable concentration for VX vapor was estimated to be 3.6 $mg/m^3$.

Kimura *et al*. (1960) tested seven subjects in what may have been the first study of the effects of IV administration to humans. The initial volunteer was Dr. Van Sim. He participated in two experiments, eighteen months apart. During the first exposure he received 4.4 µg (0.04 µg/kg) VX over a 30-second period. Three and one-half hours after the initial injection, he was administered 8.8 µg (0.08 µg/kg) VX over a 30-second period, for a combined total of 0.12 µg/kg. The reported signs and symptoms were increased airway resistance, decreased respiratory and pulse rates, frontal and retrobulbar headaches, increased MV, "conscious respiration", but no respiratory difficulty, a feeling of sweatiness, lightheadedness, and abdominal cramps. He appeared to be tired and somewhat irritable, and his speech was less clear. Blood chemistries (pH, $CO_2$, $O_2$, sugar, and lactic acid) were within normal limits. There was no effect on pupil size, and there was no consistent inhibition of RBC ChE. Eighteen months later, Dr. Sim was given 27.5 µg (0.225 µg/kg) over a 30-second period. He experienced a drop in blood pressure, an increase in MV, a 37% reduction in RBC ChE activity, frontal and retrobulbar headaches, the feeling of being "hot", and dilation of the pupils. Two hours later he was given a slow infusion of VX, at the rate of 1 µg/min over the next 3.5 hours. His RBC ChE activity fell to 15% of normal; his MV was somewhat increased; his pupils again dilated. He had a slight increase in blood pressure

and experienced visual distortions. The infusion was stopped at 3.5 hours when he suddenly became pale, stopped talking, and seemed to be "out of contact" with the observers. He then became dizzy, experienced profuse salivation, had difficulty controlling his oral musculature, began vomiting, and became irrational. ChE activity was measured in plasma, whole blood, and RBCs. There was clearly preferential inhibition of AChE, as opposed to BuChE. VX was subsequently administered via IV injection to six volunteers. Four received a 4-hour infusion of 1 µg/kg. One individual received the same dosage over a 2.5-hour period, and another individual was similarly injected over a 1.75-hour period. Maximum whole blood ChE inhibition was comparable in all individuals and ranged between about 50 and 60%. The rate of inhibition of ChE was directly proportional to the rate of administration of VX. Clinical signs and symptoms were reported to be minimal. Only one subject complained of a headache.

Lubash and Clark (1960) investigated the effects of VX placed on the volar forearm of 12 male volunteers. Four received 20 µg/kg of neat agent; four received 20 µg/kg in octylamine (1:1); four received 35 µg/kg of neat agent. ChE inhibition was significant in all individuals. Five were asymptomatic. Seven people experienced insomnia, nightmares, lightheadedness, nausea, epigastric discomfort and hyperactive bowel sounds, vomiting, and diarrhea. Four people required treatment. No significant alterations in plasma or urinary electrolytes were observed. SGOT and SGPT remained within normal limits. This was interpreted as indicating that VX was unlikely to cause liver damage. Serum amylase was normal, and the abdominal discomfort was attributed to factors other than effects on the pancreas.

Cresthull *et al.* (1963) investigated the effects of percutaneous exposure of the arm to VX vapor. The study group consisted of 29 male volunteers. Exposure areas were controlled at 500 $cm^2$ (forearm) and 1000 $cm^2$ (arm)—2.5 and 5% of the body surface, respectively. Both concentration and time were varied, with exposure concentrations ranging from 1.2 to 12.2 $mg/m^3$ and durations ranging from 5 to 75 minutes. Whole blood ChE activity was measured at 20 hours post-exposure and ranged from 100% to 24% of normal. The coefficient of correlation (r) between log Ct and per cent inhibition was 0.48. The value of $r^2$ was 0.23. This was interpreted as indicating that only 23% of the variance in the ChE inhibition was caused by changes in dosage. The remaining 77% of the variance in ChE inhibition was ascribed to other factors. These included experimental error in measuring the ChE activity, variation among individuals in skin penetration, and variation in response of the ChE activity of individuals to VX poisoning. No toxic signs or symptoms were reported. By extrapolation to the surface area of a whole body, it was estimated that the $ChE_{50}$ for an unclothed, masked man would be 141 mg $min/m^3$. However, the following caveat was applied,

> "The extrapolated value of 141 mg $min/m^3$, estimating the $ChE_{50}$, was derived from a least squares plot of widely scattered individual ChE values. This value is not very meaningful from a statistical point of view, because the 19/20 confidence limits are extremely wide, the lower limit being 35 and the upper limit being indeterminable. This 141 mg $min/m^3$ value is uncertain because it was obtained by extrapolation beyond the limits of the data and not because of the number of subjects studied."

Bramwell *et al.* (1963) performed 54 head and neck exposures on eight subjects. The individuals were exposed one at a time while standing or seated at the mouth of a tunnel down which VX vapor was flowing in an airstream at 1 m/min, at 32 $^{o}$C. Only the skin of the head and neck was exposed; the total amount of bare skin did not exceed 2000 $cm^2$. There were two exposure groups—one with respiratory protection and one without respiratory protection.

Thirty-five of the exposures were done with eyes closed and with respiratory protection. The respiratory protection was effected by placing a nose clip on the subject and having him breathe through a spirometer connected to a respirator canister. The Cts for these exposures ranged from 0.7 mg min/$m^3$ to 25.6 mg min/$m^3$. Exposure times (t) ranged from 2.25 seconds to 24 minutes (n=1), with a mean t of 5.2 minutes. The concentrations (C) ranged from 0.23 mg/$m^3$ to 5 mg/$m^3$. ChE inhibition was measurable within an hour of exposure and was greatest at 8-12 hours post-exposure. No signs or symptoms were experienced during the exposures. Despite the fact that the eyes were closed, miosis developed in nearly all subjects, beginning at least 30 minutes post-exposure and becoming maximal several hours later. It was usually accompanied or followed by fluttering and twitching of the eyelids and was more pronounced at the higher concentrations. Flushing of the skin of the head and neck was observed in 5 of the 8 subjects, and all eight individuals reported local sweating in one or more tests. Although some subjects had the perception that they were experiencing "tunnel vision" post-exposure, visual perimetry studies, following three of the exposures, were not confirmatory. Nor were there any changes in visual acuity or color vision. Five hours post-exposure, one subject developed flatulence and abdominal discomfort. An hour later he did not feel well and was experiencing waves of nausea. Eight hours post-exposure, he deteriorated rapidly and experienced severe nausea and vomiting. At this time, his RBC ChE activity was only 30% of baseline; no further inhibition occurred. Bouts of vomiting and malaise continued, and he experienced cold sweating, pallor, and a feeling of motion sickness—minus the vertigo. At 12 hours post-exposure, he was able to sleep, but experienced a nightmare shortly after falling asleep. The next morning he was fine.

Nineteen exposures were performed without respiratory protection. (These data are summarized in Table 8.) With the exception of the very first trial of the whole study, all individuals had their eyes closed. The only symptoms noted during the exposures were slight tightness in the throat and upper respiratory tract; this was not reported by all subjects. In the individual exposed with eyes open, (t = 3 minutes; C = 0.31 mg/$m^3$) miosis developed suddenly 20 to 30 minutes post-exposure and was maximal at 1.5 hours post-exposure. In the individuals exposed with eyes closed, some miosis usually developed one to three hours post-exposure. The degree of miosis was quite variable among the individuals, and appeared to be concentration-dependent. The miosis was often accompanied by a fluttering or twitching of the eyelids, almost tantamount to blepharospasm. Although the muscle effects were clearly felt by the subjects, they were not always obvious to the observers. Rhinorrhea occurred within 30 minutes of exposure in 14 of 19 trials. In four other trials, it developed more slowly. In one trial rhinorrhea was not observed. Excessive salivation, lasting for about an hour, was reported in one subject after a six-minute exposure to a concentration of 1.06 mg/$m^3$. (The authors commented that close questioning of the other subjects might have elicited further local symptoms.) Two hours post-exposure, one individual experienced some nausea and sweating. His RBC ChE activity was 60% depressed at this time. These effects abated somewhat and then recurred later when ChE inhibition had reached 70%. Several individuals also experienced malaise and lethargy and were rather unwilling to exert

themselves. Based upon all 19 trials, the inhaled dosage estimated to produce inhibition of 50% of the RBC ChE activity ($ChE_{50}$) was 13 µg/kg. However, the authors thought that apprehension had increased the subjects' MVs during the initial exposures, the first- ever inhalation trials of a very toxic chemical agent. This would have effectively increased the dosage to which the individuals were exposed and was thought to account for a relatively shallow probit slope. When these data were excluded, the estimated $ChE_{50}$ was about 8 µg/kg, which was thought to compare favorably with IV data.

Sidell (1967) reported on the IV administration of 1.3 to 1.5 µg/kg VX to 24 human volunteers. The subjects had the following signs and symptoms: dizziness, nausea, lightheadedness, malaise, weakness, nervousness, shakiness, sweating of hands and feet, blurred vision, drowsiness, difficulty concentrating, vomiting, and a feeling of being "drunk". RBC ChE activity ranged from 9 to 42% of control. No significant changes in heart rate or blood pressure were observed, however there was a decrement in the performance of the Number Facility test in the subjects receiving the highest dose.

**Table 8 Miosis in Several Species Following Sub-Acute Exposures to Low Concentrations of VX Vapor**

| VX Concentration ($mg/m^3$) | Species | # Responding | | | |
|---|---|---|---|---|---|
| | | 5-Day Exposure | | 10-Day Exposure | |
| | | Fraction | Per cent | Fraction | Per cent |
| 0.000005 | Rat | 0/12 | 0 | 3/12 | 25 |
| | Mouse | 1/12 | 8 | 4/12 | 33 |
| | Guinea Pig | 0/12 | 0 | 0/12 | 0 |
| | Rabbit | 2/6 | 33 | 0/6 | 0 |
| 0.00006 | Rat | 3/12 | 25 | 8/10 | 80 |
| | Mouse | 3/12 | 25 | 8/10 | 80 |
| | Guinea Pig | 0/10 | 0 | 0/10 | 0 |
| | Rabbit | 0/8 | 0 | 0/7** | 0 |
| 0.0002 | Rat | 9/36 | 25 | 12/21 | 56 |
| | Mouse | 21/36 | 58 | 15/22 | 68 |
| | Guinea Pig | 0/24 | 0 | 8/22 | 36 |
| | Rabbit | 8/16 | 50 | 2/16 | 13 |
| 0.004 | Rat | 14/21 | 67 | 21/27 | 78 |
| | Mouse | 10/10 | 100 | --* | -- |
| | Guinea Pig | 7/22 | 34 | 5/22 | 23 |
| | Rabbit | 8/8 | 100 | 8/8 | 100 |

*All mice dead by 5 days
**1/8 rabbits died after 5th exposure; death not agent-related

Adapted from Crook *et al.*, 1983

### 3.1.2 Animal Exposures

No long-term inhalation studies have been conducted with VX, and only one inhalation study is known (Crook *et al.*, 1983; see below) in which animals were repeatedly exposed to the agent. The stated purpose of that investigation was to establish exposure concentrations

for a chronic inhalation study to validate the control limits for VX originally proposed by McNamara *et al.* (1971). However, the chronic exposures were never done.

In the Crook *et al.* (1983) study, mice, rats, guinea pigs, and rabbits were exposed for six hours per day, five days per week, for two weeks to VX concentrations of 0.000005, 0.00006, 0.0002, or 0.004 mg/m$^3$. At the highest concentration, the animals displayed the "full spectrum of signs" of intoxication—tremors, convulsions, salivation, bloody tears, and death. The mouse was the most susceptible species; by the end of the fifth exposure to the highest concentration (0.004 mg/m$^3$) all were dead (Table 7). Miosis was the only sign of toxicity observed in any of the animals exposed to the three lower concentrations (Table 8). However, dose-dependent ChE inhibition was observed at all doses and in all species, with the exception of mice in the lowest dose group (Table 9). The estimated RBC $ChE_{50}$s, as calculated by Crook *et al.*, are given in Table 10. Recovery of ChE activity over time was not consistently observed in the rabbit, and was not observed, at all, in the rat. At the lowest exposure concentration, ChE inhibition was not significant in three species—despite clinical miosis.

Some changes in body weight were noted between control and exposed animals, but they were neither dose-related nor significant. Pathological examinations were performed following five and ten days of exposure to the two higher concentrations (0.004 and 0.0002 mg/m3). No pathological lesions were observed that could be related to VX exposure. Based upon these data Crook *et al.* (1983) predicted,

> "... animals exposed chronically to concentrations of VX vapor ranging from 0.000005 to 0.0002 mg/m$^3$ will experience slight to moderate ChE depression for the first week. Gradual recovery will occur between 5 and 10 days at rates dependent on dose and species variability.

Based upon the above it was recommended that two exposure levels be investigated in a chronic exposure study. These were 0.000001 to 0.000005 mg/m$^3$ and 0.00001 to 0.00005 mg/m$^3$. It was further stated, "In the higher level, these effects should be more pronounced....These dosages will furnish the data necessary to establish sensitivity levels for plant alarms and verify the safe exposure levels for VX presented previously by McNamara, Leitnaker, and Vocci."

The shape of the recovery curves indicated this trend would continue to the baseline values. Doses between 0.0002 and 0.004 mg/m$^3$ would cause gradual increases in depression to the point where no recovery would occur; toxic signs would appear and mortalities would be produced.

**Table 9 ChE Inhibition Following Sub-Acute Inhalation Exposure to Low Concentrations of VX Vapor**

| VX Concentration ($mg/m^3$) | Species | % ChE Depression Exposure Period | | Average Exposure Period | |
|---|---|---|---|---|---|
| | | 5 days | 10 days | 5 days | 10 days |
| 0.000005 | Mouse | 0 | 0 | | |
| | Rat | 2 | 0 | | |
| | Guinea Pig | 5 | 0 | | |
| | Rabbit | 20 | 0 | | |
| | | | | *7* | *0* |
| 0.00006 | Mouse | 31 | 0 | | |
| | Rat | 32 | 28 | | |
| | Guinea Pig | 20 | 11 | | |
| | Rabbit | 7 | 8 | | |
| | | | | *23* | *15* |
| 0.0002 | Mouse | 70 | 63 | | |
| | Rat | 55 | 56 | | |
| | Guinea Pig | 53 | 40 | | |
| | Rabbit | 40 | 24 | | |
| | | | | *55* | *46* |
| 0.004 | Mouse | --- | --- | | |
| | Rat | 78 | 77 | | |
| | Guinea Pig | 78 | 80 | | |
| | Rabbit | 75 | 76 | | |
| | | | | *77* | *78* |

Adapted from Crook *et al.*, 1983

**Table 10 Estimated RBC $ChE_{50}$ in Several Species Following Sub-Acute Exposure to Low Concentrations of VX Vapor**

| Species | Estimated RBC $ChE_{50}$ ($mg/m^3$) Exposure Period | |
|---|---|---|
| | 5 Days | 10 Days |
| | | |
| Mouse | 0.0001 | 0.0002 |
| Rat | 0.0002 | 0.0002 |
| Guinea Pig | 0.0002 | 0.0004 |
| Rabbit | 0.0004 | 0.0009 |

Adapted from Crook *et al.*, 1983

## 3.2 Traditional Approach to the Development of Exposure Criteria

The objective of traditional toxicological, non-cancer risk assessment is to establish a threshold dose below which adverse health effects are not expected to occur, or are extremely unlikely (NRC, 1993). Lehman and Fitzhugh (1954) proposed that an acceptable daily intake

(ADI) could be calculated for contaminants in human food. That concept was endorsed by the Joint FAO-WHO (Food and Agricultural Organization and World Health Organization) Expert Committee on Food Additives in 1961 and subsequently adopted by the Joint FAO-WHO Meeting of Experts on Pesticide residues in 1962 (McColl, 1990). Formally, the ADI was defined as:

**ADI = NOEL/SF**

where:

| | |
|---|---|
| NOEL | = no-observed-effect level in toxicological studies—the highest exposure level at which there are no statistically or biologically significant increases in frequency or severity of effects between the exposed population and its appropriate control |
| SF | = a safety factor to allow for variations in sensitivity to the test agent in humans, as compared with experimental animals, and for variations within the human population |

Those two sources of variation often have been accommodated by using a 10 X 10 = 100-fold SF as reviewed by the NRC's Food Protection Committee (NRC, 1970). The basic approach described above has been modified. (ADI has been relabeled by the EPA as a reference dose (RfD). Ideally, the RfD is based upon a no-adverse-effects level (NOAEL). Safety factors are now referred to as uncertainty factors (UFs), and a modifying factor (MF) has been added to account for specific scientific uncertainties in the experimental data base used to derive the RfD) (NRC, 1993). The RfD is defined by the following equation.

**RfD = NOAEL/(UF X MF)**

An adverse effect is defined as any effect that contributes to the functional impairment of an organism or that reduces the ability of the organism to respond to additional challenges (Dourson, 1986). When the data do not demonstrate a NOAEL, a LOAEL (lowest-observed-adverse-effect level) may be used. A LOAEL is defined as the lowest experimental dose at which statistically significant adverse effects occur.

Five factors may contribute to the composite UF. They are for: 1) accommodating normal human-response variability, including sensitive subgroups; 2) extrapolating from animal data to humans when human data are unavailable or inadequate; 3) accounting for uncertainties when extrapolating from a LOAEL a NOAEL; 4) extrapolating from subchronic to chronic exposure data when the latter are unavailable; and 5) extrapolating from a database that is inadequate or incomplete. An additional modifying factor (MF) may also be used to account for deficiencies not accounted for above. Factors between 1 and 10 are typically used to account for each of the sources of uncertainty (NRC, 1993).

In order to be consistent in setting exposure levels for health effects other than cancer, the EPA has adapted the oral RfD method for the estimation of inhalation reference concentrations (RfCs) (USEPA, 1994). The inhalation RfC method departs from the oral RfD paradigm by using dosimetric adjustments to scale animal exposure concentrations to human-equivalent concentrations.

Opresko (1988) discussed the application of the above RfC method to the development of AELs for chemical agents. He suggested that occupational exposure limits for nerve agents should be based on: 1) determination of the lowest observable adverse effect (LOAE), for threshold-type toxicants, such as the OP nerve agents; and 2) the direct or indirect determination of the maximum chronic exposure level which could be tolerated without producing that effect. Such levels would be used to determine no-observed-adverse effect levels (NOAELs ) in establishing health hazards criteria, if NOAELs are not determined experimentally.

## 3.3 The "Critical Adverse Effect" for VX Airborne Exposure Criteria

Current noncancer risk assessment models generally assume that 1) a population threshold exists, 2) estimates of safe exposure criteria represent subthreshold doses and 3) prevention of the "critical effect" protects against all effects. The "critical effect" is either an adverse effect or a known precursor to an adverse effect (USEPA, 1987).

"The occupational exposure limits of VX should [therefore] be based upon an evaluation of the lowest observable adverse effect and the direct or indirect determination of the maximum chronic exposure level which could be tolerated without producing that effect" (Faust and Opresko, 1988). The available data indicate the LOAE for human or animal exposure to an OP nerve agent will be a function of route of exposure.

Blood ChE inhibition has been used as the critical adverse effect in setting exposure standards (RfDs) for OP pesticides. However, its utility may be limited to identifying past exposure incidence (*i.e.*, as a biomarker of exposure in the absence of clinical effects), within a limited timeframe. It is not a good barometer of functionality or severity of intoxication, particularly following long-term, low level exposures. The Technical Panel on Risk Assessment for the EPA (Marquis, 1988), stated that, "...unequivocal correlation of a particular level of enzyme (ChE) inhibition with an observable biological effect is not well supported by either the clinical or experimental literature. The interpretation of biological significance for ChE inhibition begins initially with the point at which enzyme inhibition becomes significantly different (statistically, $p < 0.05$) from an individual baseline value or the value in a concurrent laboratory control group. The decision as to whether a statistically significant decrease in either RBC or plasma ChE activity is 'adverse' (*i.e*. of biological significance) depends upon a case by case determination. Factors in this evaluation may include dose-response relationships, comparative pharmacokinetics, and elements of study design. Statistically significant inhibition of brain AChE is an adverse effect."

## 3.4 The "Critical Study" for VX Airborne Exposure Criteria

Review of the VX exposure data given above indicates poor correlation between inhibition of blood ChE and exposure dosage or clinical signs. Indeed, clinical effects can occur in the absence of blood ChE inhibition, and there can be profound inhibition of blood ChE, with relatively few clinical signs or symptoms. Further, the model upon which the existing criteria for VX are based is dependent upon recovery of BuChE, and VX preferentially inhibits AChE.

The data also underscore the importance of route of exposure. Following vapor exposure to nerve agents, the sign most frequently occurring, at the lowest doses, in humans and animals is miosis, and it can occur in the absence of blood ChE inhibition (Rubin *et al.*, 1957; Mioduszewski *et al.*, 1998). Following administration by other routes of exposure, miosis occurs only at very high doses.

Preferably, chronic human inhalation data would be used in establishing AEL guidelines. However, the only available human inhalation data are those of Bramwell *et al.* (1963), which are limited to very short exposure durations. The study is further limited by the fact that both concentration and time were varied—there were essentially no replicated doses. In addition, the VX was dispersed with benzene, and there is uncertainty about the dosages that the subjects actually received. All but one individual had his eyes closed. Nonetheless, most of the subjects developed miosis. Interestingly, the eyes-open exposure appears to have been the first of 54 total trials. One can only speculate as to why the 53 other trials were done with eyes closed, and the following quote fuels the speculation.

> "Plans to determine the minimum exposure to cause miosis could not be proceeded with as miosis developed after exposure to quite low Cts even when the eyes were closed throughout exposure."

The only repeated dose vapor inhalation study that could be found was that done by Crook *et al.* (1983). The animals were exposed to very low concentrations of VX vapor for six hours per day, five days per week, for two weeks. The purpose of the study was to validate the control limits for VX originally proposed by McNamara *et al.* in 1971. The conclusions and recommendations of the Crook *et al.* (1983) report were as follows:

> "Based upon the results of the 10-day subacute studies, two exposure levels are proposed for chronic VX studies: 0.000001 to 0.000005 $mg/m^3$ and 0.00001 to 0.00005 $mg/m^3$. In the higher level, these effects should be more pronounced."

The existing criteria for the general population and workers are 0.000003 $mg/m^3$ and 0.00001 $mg/m^3$, respectively. It is clear that, following chronic exposure, Crook *et al.* expected to see effects at concentration levels at, near, or less than the current criteria. However, it is not possible to verify the exposure concentrations actually achieved by Crook *et al.* in that study. As stated by Crook *et al.*, "The average concentrations of VX vapor determined for the four levels over 10-day exposure periods…may vary by an order of magnitude because of sampling techniques and possible trace bleach contamination. Other variations in the concentrations were due mainly to slight fluctuations in water-bath temperatures and the nitrogen pressures and flows routinely encountered when generating trace concentrations of VX vapor."

In the Bramwell *et al.* (1963) study, organic solvents were used to help disperse the agent. Further, the subjects were seated or standing in front of a "tunnel" down which the vapor flowed. In short, the individuals were not in a rigorously controlled agent atmosphere.

Although there are instances in the IRIS database (USEPA, 1988, 1992, 1993), in which RfD values have been derived from acute or subacute human exposures to OP pesticides,

such data are less than ideal for developing chronic toxicity estimates. Given the uncertainty in the actual exposure concentrations, neither the Bramwell *et al.* (1963) nor Crook *et al.* (1983) study can be selected as the critical study. Nonetheless, since no other VX inhalation studies, suitable for deriving AELs were identified, it was deemed important to determine the AELs that would be calculated if these data were used. Those calculations are given below.

The alternative procedure that was employed for each of the recommended limits developed in this herein was based on the estimated relative potency of VX to GB. A potency ratio of 10 was selected, based upon effective dosages for miosis and mild effects for airborne agent (IDA, 1998). The basis of this value is as follows. Reutter and Wade (1994) endorsed the existing estimate of 0.09 mg min/$m^3$, for miosis and mild effects secondary to airborne VX (2-10 minute exposure), but recommended lowering the estimate for GB to 0.5 mg min/$m^3$ (2-10 minute exposure). The COT (1997) suggested that the GB estimate should be higher than that put forward by Reutter and Wade (see Table 3). The participants in the 1988 IDA Workshop on Chemical Agent Toxicity* agreed to recommend an estimate of 1 mg min/$m^3$ for GB and to round the estimate for VX to 0.1 mg min/$m^3$, so as not to indicate a level of precision that does not exist. Precedence for this relative potency approach is found in the G-agent Criteria document by Mioduszewski *et al*. (1998). In that document, the exposure criteria for GA, GD, and GF were based upon the respective estimated relative potencies of those agents to GB. This was done because GB is the only G-type organophosphonate nerve agent for which there are sufficient data to establish inhalation exposure criteria.

### 3.4.1 Derivation of the WPL for VX Vapor

#### 3.4.1.1 WPL Based Upon Subacute Animal Data

It has been demonstrated (Brain and Mensah, 1983) that exposure concentration is not an adequate description of lung dose for different species and that the lung dose is independent of body size. Therefore, for the calculation of human exposure criteria from animal data, the experimental exposure concentrations for the animal species are adjusted to "human equivalent" concentrations according to the following formula:

$$\mathbf{HEC = (R_{Animal} \times C_{Animal} \times BW_{Human})/(R_{Human} \times BW_{Animal})}$$

where:

| | |
|---|---|
| HEC | = human equivalent exposure concentration (mg/$m^3$) |
| $R_{ANIMAL}$ | = animal respiratory volume ($m^3$/day) |
| $C_{ANIMAL}$ | = animal exposure concentration (mg/$m^3$) |
| $BW_{HUMAN}$ | = human body weight (kg) |
| $R_{HUMAN}$ | = human respiratory volume ($m^3$/day) |
| $BW_{ANIMAL}$ | = animal body weight (kg) |

When more-or-less equivalent data exist for multiple species, the USEPA (1994) has stated,

"For RfCs, the most sensitive species is designated as the species that shows the critical adverse effect at an exposure level that, when dosimetrically adjusted, results in the lowest HEC [human equivalent concentration]."

In the data of Crook *et al.* (1983), following 10 days of exposure, miosis was observed in both rats and mice at the lowest concentration tested (0.000005 mg/$m^3$).

For the mouse:

$$\mathbf{HEC = (R_{Animal} \times C_{Animal} \times BW_{Human})/(R_{Human} \times BW_{Animal})}$$

where:

| | |
|---|---|
| HEC | = Human equivalent exposure concentration (mg/$m^3$) |
| $R_{ANIMAL}$ | = Mouse respiratory volume = 0.04 $m^3$/day |
| $C_{ANIMAL}$ | = Mouse exposure concentration = 0.000005 mg/$m^3$ |
| $BW_{HUMAN}$ | = Human body weight = 70 kg |
| $R_{HUMAN}$ | = Human respiratory volume = 20 $m^3$/day |
| $BW_{ANIMAL}$ | = Mouse body weight = 0.0275 kg |

$$\mathbf{HEC = (0.04\ m^3/day \times 0.000005\ mg/m^3 \times 70\ kg)/(20\ m^3/day \times 0.0275\ kg)}$$

$$\mathbf{HEC = 0.000025454\ mg/m^3}$$

For the rat:

$$\mathbf{HEC = (R_{Animal} \times C_{Animal} \times BW_{Human})/(R_{Human} \times BW_{Animal})}$$

where:

| | |
|---|---|
| HEC | = Human equivalent exposure concentration (mg/$m^3$) |
| $R_{ANIMAL}$ | = Rat respiratory volume = 0.2 $m^3$/day |
| $C_{ANIMAL}$ | = Rat exposure concentration = 0.000005 mg/$m^3$ |
| $BW_{HUMAN}$ | = Human body weight = 70 kg |
| $R_{HUMAN}$ | = Human respiratory volume = 20 $m^3$/day |
| $BW_{ANIMAL}$ | = Rat body weight = 0.425 kg |

$$\mathbf{HEC = (0.2\ m^3/day \times 0.000005\ mg/m^3 \times 70\ kg)/(20\ m^3/day \times 0.425\ kg)}$$

$$\mathbf{HEC = 0.000008235\ mg/m^3}$$

The rat is the more sensitive species.

The $LOAEL_{HEC}$ is applied to the following formula for calculating the AEL for occupational exposures.

$$\mathbf{WPL = LOAEL_{HEC} \times (Resp_{DATA}/Resp_{OCCUP}) \times (Exp_{DATA}/Exp_{OCCUP}) \times [1/(UF_X \times MF)]}$$

where:

| | |
|---|---|
| WPL | = Allowable ambient air concentration for work place ($mg/m^3$) |
| $LOAEL_{HEC}$ | = Lowest observed adverse effect level (HEC) |
| | = 0.000008 ($mg/m^3$) |
| $Resp_{DATA}$ | = Experimental (resting) respiratory volume (10 L/min) |
| $Resp_{OCCUP}$ | = Occupational respiratory volume (20.8 L/min) |
| $Exp_{OCCUP}$ | = Occupational exposure (480 min/day x 5 days/week) |
| $Exp_{DATA}$ | = Experimental exposure (360 min/day x 5 days/week) |
| $UF_X$ | = Product of uncertainty factors ($UF_H$ x $UF_A$ x $UF_S$ x $UF_L$ x $UF_D$) |
| MF | = Modifying factor |

**$WPL = 0.000008 \times (10 / 20.8) \times (360 / 480) \times [1 / (3 \times 10 \times 3 \times 10)]$**

**$WPL = 0.000000003\ mg/m^3$**

Uncertainty Factors (UF):

| | |
|---|---|
| $UF_H$ | = 1 (average human to sensitive human population) |
| $UF_A$ | = 3 (animal to human extrapolation) |
| $UF_S$ | = 10 (sub-acute to chronic exposure extrapolation) |
| $UF_L$ | = 3 (LOAEL to NOAEL extrapolation) |
| $UF_D$ | = 10 (incomplete data) |
| MF | = 1 (not necessary) |

A value of 1 was selected for $UF_H$ because the occupational population is screened to exclude sensitive subpopulations. A value of 3 was selected for $UF_A$ because humans are considered the most sensitive species. (There are no data indicating humans are less sensitive than the experimental animal species, and for the organophosphate nerve agent GB, humans are clearly more sensitive than other species.) A value of 10 was selected for $UF_S$ because the data were from a sub-acute exposure, and there was no recovery from miosis during the exposure period; in fact, it was maximal at the end of the study. A value of 3 was used for $UF_L$ because the level of effect (miosis) was minimal. A value of 10 was used for $UF_D$ because the database is minimal, of dubious quality, and does not include any chronic data or an adequate determination of carcinogenic potential. A value of 1 was chosen for the MF.

#### 3.4.1.2 WPL Based Upon Acute Human Data

Bramwell *et al.* (1963) did not enumerate the specific clinical signs and symptoms for each individual, for each exposure. Miosis occurred in "most", despite closed eyes; therefore, the dosage to the eyes was unknown (with one exception). Rhinorrhea was reported in 14 of 19 of the vapor inhalation exposures.

Another difficulty in using these data is that both time and concentration were varied. The lowest concentrations were those used for the seven, 3-minute exposures. Based upon the data given above, one must infer that there were signs in several of these individuals. The dosages for these exposures ranged from 0.6 mg $min/m^3$ to 1.7 mg $min/m^3$, with a mean of 1.1 mg $min/m^3$. This is more than a factor of two difference in total dosage, and VX is a very potent

chemical agent. [The estimated effective dosage for miosis in military personnel is 0.09 mg min/$m^3$ for a 2- to 10-minute exposure**.** (See Table 3).]

Bramwell *et al.* (1963) measured respiratory MV in seven of their PC vapor exposure trials. The mean was 16.8 L/min. Using this MV and 0.4 mg/$m^3$, the mean exposure concentration for the seven, 3-minute exposures, the formula for calculating the WPL would be

$$\mathbf{WPL = LOAEL \times (Resp_{DATA}/Resp_{OCCUP}) \times (Exp_{DATA}/Exp_{OCCUP}) \times [1/(UF_X \times MF)]}$$

where:

| | |
|---|---|
| WPL | = Allowable ambient air concentration for work place (mg/$m^3$) |
| LOAEL | = Lowest observed adverse effect level = 0.4 (mg/$m^3$) |
| $Resp_{DATA}$ | = Experimental (resting) respiratory volume (16.8 L/min) |
| $Resp_{OCCUP}$ | = Occupational respiratory volume (20.8 L/min) |
| $Exp_{OCCUP}$ | = Occupational exposure (480 min/day x 5 days/week) |
| $Exp_{DATA}$ | = Experimental exposure (3 min x 1 day) |
| $UF_X$ | = Product of uncertainty factors ($UF_H \times UF_A \times UF_S \times UF_L \times UF_D$) |
| MF | = Modifying factor |

$$\mathbf{WPL = 0.4 \times (16.8 / 20.8) \times (3 / 2400) \times [1 / (10 \times 10 \times 10)]}$$

$$\mathbf{WPL = 0.0000004\ mg/m^3}$$

Uncertainty Factors (UF):

| | |
|---|---|
| $UF_H$ | = 1 (average human to sensitive human population) |
| $UF_A$ | = 1 (animal to human extrapolation) |
| $UF_S$ | = 10 (acute to chronic exposure extrapolation) |
| $UF_L$ | = 10 (LOAEL to NOAEL extrapolation) |
| $UF_D$ | = 10 (incomplete data) |
| MF | = 1 (not necessary) |

A value of 1 was selected for $UF_H$ because the occupational population is screened to exclude sensitive subpopulations. A value of 1 was selected for $UF_A$ because humans are considered the most sensitive species. A value of 10 was selected for $UF_S$ because the data were from an acute exposure. A value of 10 was used for $UF_L$ because ChE depression was observed in addition to miosis. Further, Bramwell *et al.* (1963) were unable to determine the minimum concentration to produce miosis, and for GB, miosis is produced at concentrations lower than those producing ChE inhibition. A value of 10 was chosen for $UF_D$ because the number of subjects was extremely limited, and several experimental parameters were varied, and the total database for VX is inadequate. A value of 1 was selected for the MF.

As discussed, the quality of the Bramwell *et al.* (1963) acute human study and the Crook *et al.* (1983) subacute animal study is considered inadequate for determining chronic exposure limits. The inherent uncertainties of these studies undermine the confidence in a WPL derived from them. Nonetheless, it is noted that the available VX IH data do not support the existing WPL.

### 3.4.1.3 WPL Based Upon the Estimated Relative Potency of VX to GB

In the absence of better data, it is recommended that the WPL for VX should be based upon the estimated relative 10-fold greater miotogenic potency of VX, as compared with GB. (Miosis is one of the mildest adverse health effects produced by the OP nerve agents.) It is noted, however, that unlike GB, VX presents a significant percutaneous vapor hazard, and the potency ratio of VX to GB for this endpoint is estimated to be about 120. It is hypothesized that the threshold for percutaneous vapor effects is higher than that for miosis, but this relationship has not been well characterized, particularly for long exposures to low concentrations.

The WPL for GB, as recommended by Mioduszewski *et al.* (1998), is 0.0001 $mg/m^3$. Thus, the WPL for VX is calculated as follows.

$$\mathbf{WPL_{VX} = WPL_{GB} / RP}$$

$$\mathbf{WPL_{VX} = 0.0001 / 10}$$

$$\mathbf{WPL_{VX} = 0.00001\ mg/m^3}$$

where:

| | |
|---|---|
| WPL | = Allowable ambient air concentration for work place ($mg/m^3$) |
| RP | = Estimated relative miotogenic potency of VX to GB |

This value is equivalent to the existing WPL (DHHS, 1988) and is 25 to 3000-fold higher than the WPLs derived from the human and animal data, respectively.

### 3.4.2 Derivation of the GPL for VX Vapor

### 3.4.2.1 GPL Based Upon Subacute Animal Data

The approach used to calculate the general population limit (GPL) is the same as that used for the WPL. The critical study, HEC, and LOAEL are applied to the same formula. The only differences are the respiratory volume, exposure period, and uncertainty factors appropriate for the general population.

Based upon the sub-acute rat data, the GPL is calculated according to the following formula:

$$\mathbf{GPL = LOAEL_{HEC} \times (Resp_{DATA}/Resp_{POP}) \times (Exp_{DATA}/Exp_{POP}) \times [1/(UF_X \times MF)]}$$

where:

| | |
|---|---|
| GPL | = Allowable ambient air concentration for general population ($mg/m^3$) |
| $LOAEL_{HEC}$ | = Lowest observed adverse effect level (HEC) = 0.000008 ($mg/m^3$) |
| $Resp_{DATA}$ | = Experimental (resting) respiratory volume (10 L/min) |
| $Resp_{POP}$ | = Population respiratory volume (13.9 L/min) |
| $Exp_{POP}$ | = Population exposure (1440 min/day x 7 days/week) |
| $Exp_{EXPTL}$ | = Experimental exposure (360 min/day x 5 days/week) |
| $UF_X$ | = Product of uncertainty factors ($UF_H$ x $UF_A$ x $UF_S$ x $UF_L$ x $UF_D$) |
| MF | = Modifying factor |

**$GPL = 0.000008 \times (10 / 13.9) \times (1800 / 10080) \times [1 / (3000^*)]$**

**$GPL = 0.0000000003\ mg/m^3$**

Uncertainty Factors (UF):

| | |
|---|---|
| $UF_H$ | = 10 (average human to sensitive human population) |
| $UF_A$ | = 3 (animal to human extrapolation) |
| $UF_S$ | = 10 (sub-acute to chronic exposure extrapolation) |
| $UF_L$ | = 3 (LOAEL to NOAEL extrapolation) |
| $UF_D$ | = 10 (incomplete data) |
| MF | = 1 (not necessary) |

*When the total value of the uncertainty factors is 10,000, the USEPA (1994) recommends the use of a combined uncertainty factor of 3,000 to account for overlap of the different uncertainty categories. Thus, the uncertainty factors are shown collectively in the above formula as 3000.

A value of 10 was chosen for $UF_H$ because the general population includes sensitive subpopulations. A value of 3 was selected for $UF_A$ because humans are considered the most sensitive species. A value of 10 was selected for $UF_S$ because the data were from a sub-acute exposure, and there was no recovery from miosis during the exposure period; in fact, it was maximal at the end of the study. A value of 3 was used for $UF_L$ because the level of effect (miosis) was minimal. A value of 10 was used for $UF_D$ because the database is minimal, of questionable quality, and does not include any chronic data or an adequate determination of carcinogenic potential. A value of 1 was chosen for the MF.

### 3.4.2.2 GPL Based Upon Acute Human Data

Based upon the Bramwell *et al.* (1963) data, the GPL would be calculated as follows.

**$GPL = LOAEL \times (Resp_{DATA}/Resp_{POP}) \times (Exp_{DATA}/Exp_{POP}) \times [1/(UF_X \times MF)]$**

where:

| | |
|---|---|
| GPL | = Allowable ambient air concentration for general population ($mg/m^3$) |
| LOAEL | = Lowest observed adverse effect level = 0.4 ($mg/m^3$) |
| $Resp_{DATA}$ | = Experimental (resting) respiratory volume (16.8 L/min) |
| $Resp_{POP}$ | = Population respiratory volume (13.9 L/min) |
| $Exp_{POP}$ | = Population exposure (1440 min/day x 7 days/week) |
| $Exp_{DATA}$ | = Experimental exposure (3 min x 1 day) |
| $UF_X$ | = Product of uncertainty factors ($UF_H$ x $UF_A$ x $UF_S$ x $UF_L$ x $UF_D$) |
| MF | = Modifying factor |

**$GPL = 0.4 \times (16.8 / 13.9) \times (3 / 10080) \times [1 / (3000)^*]$**

**$GPL = 0.00000005\ mg/m^3$**

Uncertainty Factors (UF):

| | |
|---|---|
| $UF_H$ | = 10 (average human to sensitive human population)* |
| $UF_A$ | = 1 (animal to human extrapolation) |
| $UF_S$ | = 10 (acute to chronic exposure extrapolation) |
| $UF_L$ | = 10 (LOAEL to NOAEL extrapolation) |
| $UF_D$ | = 10 (incomplete data) |
| MF | = 1 (not necessary) |

*When the total value of the uncertainty factors is 10,000, the USEPA (1994) recommends the use of a combined uncertainty factor of 3,000 to account for overlap of the different uncertainty categories. Thus, the uncertainty factors are shown collectively in the above formula as 3000.

A value of 10 was selected for $UF_H$ because the general population includes sensitive subpopulations. A value of 1 was selected for $UF_A$ because humans are considered the most sensitive species. A value of 10 was selected for $UF_S$ because the data were from an acute exposure. A value of 10 was used for $UF_L$ because ChE depression was observed in addition to miosis. Further, Bramwell *et al.* (1963) were unable to determine the minimum concentration to produce miosis, and for GB, miosis is produced at concentrations lower than those producing ChE inhibition. A value of 10 was chosen for $UF_D$ because the number of subjects was extremely limited, and several experimental parameters were varied, and the total database for VX is inadequate. A value of 1 was selected for the MF.

As stated above, the quality of Bramwell *et al.* (1963) acute human study and the Crook *et al.* (1983) subacute animal study is considered inadequate for determining chronic exposure limits. The inherent uncertainties of these studies undermine the confidence in a GPL so-derived. Nonetheless, it is noted that the available VX IH data do not support the existing GPL.

### 3.4.2.3 GPL Based Upon the Estimated Relative Potency of VX to GB

As stated, previously, in the absence of better data, it is recommended that the WPL for VX should be based upon the estimated relative 10-fold greater miotogenic potency of VX, as compared with GB. Again, it is noted that unlike GB, VX presents a significant percutaneous vapor hazard, and the potency ratio of VX to GB for this endpoint is estimated to be about

120. It is hypothesized that the threshold for percutaneous vapor effects is higher than that for miosis, but this relationship has not been well characterized, particularly for long exposures to low concentrations.

The GPL for GB, as recommended by Mioduszewski *et al.* (1998), is 0.000003 $mg/m^3$. Thus, the GPL for VX is calculated as follows.

$$\mathbf{GPL_{VX} = GPL_{GB} / RP}$$

$$\mathbf{GPL_{VX} = (0.000003\ mg/m^3) / 10}$$

$$\mathbf{GPL_{VX} = 0.0000003\ mg/m^3}$$

where:

| | |
|---|---|
| GPL | = Allowable ambient air concentration for general population ($mg/m^3$) |
| RP | = Estimated relative miotogenic potency of VX to GB |

This value is 10-fold lower than the existing GPL (DHHS, 1988), and is 6- and 1000-fold higher than the GPLs derived from the human and animal data, respectively.

### 3.4.3 The Short-Term Exposure Limit (STEL) for VX Vapor

The American Conference of Government Industrial Hygienists (ACGIH) defines STEL as "a 15-minute time weighted average (TWA) exposure which should not be exceeded at any time during a workday even if the 8-hour TWA is within the threshold limit value (TLV)-TWA." It is further stated that exposures above the TLV-TWA up to the STEL should be no longer than 15 minutes and should not occur more than four times per day. There should be at least 60 minutes between successive exposures in this range. An averaging period other than 15 minutes may be recommended when this is warranted by observed biological effects."

#### 3.4.3.1 STEL Based Upon Acute Human Data

The only set of available data from which a STEL could potentially be calculated is that of Bramwell *et al.* (1963). However, the confidence in these data is low, and the exposure durations were shorter than those defined for a typical STEL.

If the averaged 3-minute data from the Bramwell *et al.* (1963) study are adjusted for a 60 minute exposure duration (15 minutes repeated up to 4 times per day):

$$\mathbf{STEL = LOAEL \times (Resp_{DATA}/Resp_{OCCUP}) \times (Exp_{DATA}/Exp_{OCCUP}) \times [1/(UF_X \times MF)]}$$

where:

| | |
|---|---|
| STEL | = Short-term exposure limit for the work place ($mg/m^3$) |
| LOAEL | = Lowest observed adverse effect level = 0.4 ($mg/m^3$) |
| $Resp_{DATA}$ | = Experimental (resting) respiratory volume (16.8 L/min) |
| $Resp_{OCCUP}$ | = Occupational respiratory volume (20.8 L/min) |
| $Exp_{OCCUP}$ | = Occupational exposure (15 min x 4)/day) x 5 days/week |
| $Exp_{DATA}$ | = Experimental exposure (3 min x 1)/day) |
| $UF_X$ | = Product of uncertainty factors (UFH x UFA x UFS x UFL x UFD) |
| MF | = Modifying factor |

**STEL = 0.4 x (16.8 / 20.8) x [3 / (60 x 5)] [1 / (3 x 10 x 10)]**

**STEL = 0.00005 $mg/m^3$**

Uncertainty Factors (UF):

| | |
|---|---|
| $UF_H$ | = 1 (average human to sensitive human population) |
| $UF_A$ | = 1 (animal to human extrapolation) |
| $UF_S$ | = 3 (acute to chronic exposure extrapolation) |
| $UF_L$ | = 10 (LOAEL to NOAEL extrapolation) |
| $UF_D$ | = 10 (incomplete quality data) |
| MF | = 1 (not necessary) |

A value of 1 was selected for $UF_H$ because sensitive subpopulations are not included. A value of 1 was selected for $UF_A$ because humans are considered the most sensitive species. A value of 3 was selected for $UF_S$ because the data were from an acute exposure, but a STEL is of a less chronic nature than a WPL. A value of 10 was used for $UF_L$ because ChE depression was observed in addition to miosis. Further, Bramwell *et al.* (1963) were unable to determine the minimum concentration to produce miosis, and for GB, miosis is produced at concentrations lower than those producing ChE inhibition. A value of 10 was chosen for $UF_D$ because the number of subjects was extremely limited; several experimental parameters were varied, and the total database for VX is inadequate. A value of 1 was selected for the MF.

### 3.4.3.2 STEL Based Upon the Estimated Relative Potency of VX to GB

Considering the shortcomings of the Bramwell *et al.* (1963) study and remaining consistent with the procedure used to derive the long-term exposure limits, it is recommended that the STEL for VX be based upon the estimated relative tenfold greater miotogenic potency of VX, as compared with GB.

The STEL for GB, as recommended by Mioduszewski *et al.* (1998; *Erratum, 2000*) is 0.0004 $mg/m^3$. Thus, the STEL for VX is calculated as follows.

$$\mathbf{STEL_{VX} = STEL_{GB} / RP}$$

$$\mathbf{STEL_{VX} = (0.0004\ mg/m^3) / 10}$$

$$\mathbf{STEL_{VX} = 0.00004\ mg/m^3}$$

where:

| | |
|---|---|
| STEL | = Allowable ambient concentration for work force for up to 4 incursions of no more than 15 minutes each |
| RP | = Estimated relative miotogenic potency of VX to GB |

### 3.4.4 The Immediately Dangerous to Life and Health (IDLH) Concentration for VX Vapor

The current NIOSH definition for an immediately dangerous to life or health condition (NIOSH, 1997) is a situation "that poses a threat of exposure to airborne contaminants when that exposure is likely to cause death or immediate or delayed permanent adverse health effects or prevent escape from such an environment." It is also stated that the purpose of establishing an IDLH is to "ensure that the worker can escape from a given contaminated environment in the event of failure of the respiratory protection equipment."

#### 3.4.4.1 The Current IDLH

Two IDLH estimates were found for VX. For personnel conducting emergency operations or operations in unknown but potentially high agent concentrations, DA PAM 40-8 (1990) states,

> "a. A NIOSH/MSHA-approved, pressure demand full facepiece SCBA or supplied-air respirator suitable for use in high agent concentrations with protective ensemble.
>
> "b. During emergency operations, use the best available respiratory protection and personnel ensemble. If protection in A above, is not available, a full facepiece, chemical canister, air-purifying protective mask with hood is acceptable..."

The concentration of VX for which the above is applicable is >0.02 mg/m$^3$. Although this is effectively an IDLH, the exposure scenario is not limited to 30 minutes, nor does it exclude irreversible health effects.

An OTSG Memorandum (1991) states that 0.02 mg/m$^3$ is the IDLH for VX. This memorandum references a table in an enclosure, which contains a paper entitled, "Establishing Concentrations Immediately Dangerous to Life or Health (IDLH) for Agents, GA, GB, GD, VX, HD, and L". The document is accompanied by USAEHA memorandum dated, 12 April 1991, stating that the paper was written as a scientifically defensible article. The IDLH given therein for VX is 0.04 mg/m$^3$. It was derived as follows.

"As with GD, the literature with regard to the human effects from this chemical is also scarce. Most predictions have been derived from animal IV work. VX has been shown to have a direct effect on the eye, producing miosis to a 25 times greater extent than GB. But miosis is a reversible effect and would not impede escape, given normal lighting. Watson's table [Watson *et al.*, 1989] on the comparative acute toxicity of warfare agents to humans shows that when compared with GB, the inhalation toxicity increases with VX by 2.3 to 2.8 times. McNamara *et al*. (1973) used a factor of 2.3. For the sake of this work, we will use a factor of 3 and begin our calculations...using the 2 minute ECt of 15 mg min/$m^3$ [for GB] (MV = 10) which results in runny nose, tightness of chest, headache, and miosis. Since we are uncertain as to what the comparable ECt for VX at 30 minutes would be, we are leaving the 30 minute value the same as the 2 minute value, to remain conservative (it is reasonable to expect that the 30 minute ECt would be higher than 15 mg min/$m^3$."

"Converting the MV to 42 L/min:
(15 mg min/m3 x 10 L/min) / (42 L/min) = 3.6 mg min/$m^3$

"To obtain the comparable VX value, we use a factor of three:
(3.6 mg min/$m^3$)/3 = 1.2 mg min/$m^3$

"Converting the 30 min ECt to the EC:
1.2 mg min/$m^3$/30 min = 0.04 mg/$m^3$."

The most recent "official" document giving an estimated IDLH for VX is the 1997 version of AR 385-61 (DA, 1997b). The IDLH for VX is given as 0.02 mg/$m^3$.

### 3.4.4.2 The Proposed IDLH

The derivation and rationale for the VX IDLH estimate of 0.02 mg/$m^3$ is undocumented. The estimate of 0.04 mg/$m^3$ is difficult to defend because it was predicated upon a GB estimate that is not supported by the available data (Reutter and Wade, 1994; COT, 1997).

The only known data on human IH exposure to VX vapor are those of Bramwell *et al.* (1963) (see above). Most of the exposures were done with eyes closed, and both exposure concentration and duration were varied, so their utility for deriving an IDLH is considerably compromised. Mioduszewski *et al.* (1998) have recently proposed an IDLH estimate of 0.1 mg/$m^3$ for GB. This value was calculated to adjust for the facts that the salient human data were derived from exposures of male subjects; there are women in the workplace, and females may be more sensitive than males.

Given the rationale used throughout this document, the proposed IDLH for VX is based upon the estimated relative potency of VX to GB for mild effects. The effective dosages for severe effects are not significantly different from those for lethality, and hence, the estimated relative potency for severe effects cannot be used.

$$IDLH_{VX} = IDLH_{GB} / RP$$

$$IDLH_{VX} = 0.1 / 10$$

$$IDLH_{VX} = 0.01\ mg/m^3$$

where:

| | |
|---|---|
| IDLH | = The maximum airborne concentration from which, in the event of respirator failure, one could escape within 30 minutes without a respirator and without experiencing any escape-impairing (*e.g.* severe eye irritation) or irreversible health effects. |
| RP | = Estimated relative potency of VX to GB for mild effects |

### 3.4.5 General Population Category 1 Acute Exposure Guideline Levels (AEGL-1s) for VX Vapor

According to the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL,1996), AEGLs represent short-term threshold or ceiling exposure values intended for the protection of the general public—including susceptible or sensitive individuals, but not hypersusceptible or hypersensitive individuals. The AEGLs represent biological reference values for this defined human population, and they are developed for exposure periods of 30 min, 1 hr, 4 hr, and 8 hr. The AEGL-1 biological endpoint is the airborne concentration (expressed as ppm or $mg/m^3$) of a substance at or above which it is predicted that the general population, including "susceptible" but excluding "hypersusceptible" individuals, could experience notable discomfort. Airborne concentrations below the AEGL-1 represent exposure levels that may produce mild odor, taste or other sensory irritations. AEGLs may be adapted by Federal and State agencies for chemical emergency programs.

#### 3.4.5.1 AEGLs Based Upon Human Data

The data of Bramwell *et al.* (1963) are the only known human data suitable for calculating AEGLs.

$$AEGL\text{-}1_{30} = LOAEL \times (Resp_{DATA}/Resp_{POP}) \times (Exp_{DATA}/Exp_{POP}) \times [1/(UF_X \times MF)]$$

where:

| | |
|---|---|
| $AEGL\text{-}1_{30}$ | = Allowable concentration in ambient air for the general population, for a 30-minute exposure |
| LOAEL | = Lowest observed adverse effect level (HEC) = 0.4 ($mg/m^3$) |
| $Resp_{data}$ | = Experimental (resting) respiratory volume (16.8 L/min) |
| $Resp_{pop}$ | = Population respiratory volume (13.9 L/min) |
| $Exp_{data}$ | = Experimental exposure (3 min/day x 1 day) |
| $Exp_{pop}$ | = Emergency exposure (30, 60, or 240 min/day x 1day) |
| $UF_X$ | = Product of uncertainty factors ($UF_H \times UF_A \times UF_S \times UF_L \times UF_D$) |
| MF | = Modifying factor |

$$\mathbf{AEGL\text{-}1_{30} = 0.4 \times (16.8/13.9) \times (3/30) \times [1/(10 \times 10)]}$$

$$\mathbf{AEGL_{30} = 0.0005\ mg/m^3}$$

Similarly for 1-hour and 4-hour exposure durations

$$\mathbf{AEGL\text{-}1_{60} = 0.4 \times (16.8/13.9) \times (3/60) \times [1/(10 \times 10)]}$$

$$\mathbf{AEGL_{60} = 0.0002\ mg/m^3}$$

$$\mathbf{AEGL\text{-}1_{240} = 0.4 \times (16.8/13.9) \times (3/240) \times [1/(10 \times 10)]}$$

$$\mathbf{AEGL_{240} = 0.00006\ mg/m^3.}$$

Uncertainty Factors (UF):

| | |
|---|---|
| $UF_H$ | = 10 (average human to sensitive human population) |
| $UF_A$ | = 1 (animal to human extrapolation) |
| $UF_S$ | = 1 (acute to chronic exposure extrapolation) |
| $UF_L$ | = 1 (LOAEL to NOAEL extrapolation) |
| $UF_D$ | = 10 (incomplete data) |
| MF | = 1 (not necessary) |

A value of 10 was selected for $UF_H$ because sensitive subpopulations are included. A value of 1 was selected for $UF_A$ because humans are considered the most sensitive species. A value of 1 was selected for $UF_S$ because the data were from an acute exposure. A value of 1 was used for $UF_L$ because level of effect observed experimentally was comparable to what is being estimated. A value of 10 was chosen for $UF_D$ because the number of subjects was extremely limited; several experimental parameters were varied, and the total database for VX is inadequate. A value of 1 was selected for the MF.

### 3.4.5.2 AEGLs Based Upon the Estimated Relative Potency of VX to GB

Alternatively, AEGLs could be based upon the estimated relative potency of VX to GB and calculated from the proposed AEGLs for GB (Mioduszewski *et al.*, 1998).

$$\mathbf{AEGL_{VX} = AEGL_{GB} / RP}$$

$$\mathbf{AEGL\text{-}1_{30} = (0.0024\ mg/m^3) / 10}$$

$$\mathbf{AEGL\text{-}1_{30} = 0.0002\ mg/m^3}$$

Similarly for 1-hour and 4-hour exposure durations

$$\mathbf{AEGL\text{-}1_{60} = (0.0012) / 10}$$

$$\mathbf{AEGL\text{-}1_{60} = 0.0001\ mg/m^3}$$

$$\mathbf{AEGL\text{-}1_{240} = (0.0003)}$$

$$\mathbf{AEGL_{240} = 0.00003\ mg/m^3}$$

## 4. CONCLUSIONS: Existing *vs.* Recommended Criteria

The existing and recommended criteria for VX are summarized in Table 11. The existing WPL and GPL exposure criteria were originally promulgated by McNamara *et al*. in 1973 and were based upon *estimated* effective dosages for VX and a model derived for the development of similar criteria for GB. Few, if any IH data for VX were known to exist, and many of the estimated effective dosages for VX were *calculated* from estimates for GB. In turn, some of the fundamental estimates for GB were based upon routes of exposure other than inhalation, although it was clearly recognized that miosis: (1) was an unacceptable endpoint, (2) resulted from direct local effects of vapor on the eye, (3) could occur in the absence of any detectable ChE inhibition, and (4) was one of the earliest signs resulting from airborne exposure. The existing IDLHs were developed in the early 1990s, and the methods and rationale used to derive them are either unknown or are not supported by the available data.

Since that time, some acute human inhalation data have been made available (Bramwell *et al.*, 1963), and a sub-acute animal study has been performed (Crook *et al.*, 1983). These are the only known VX IH data appropriate for deriving these types of exposure criteria. Overall, the data for VX are sparse; few studies have employed airborne exposures, and chronic studies have not been done by any exposure route.

Ironically, the animal study (Crook *et al.*, 1983) was done to establish exposure concentrations for a chronic VX IH study to validate the existing VX criteria. However, miosis occurred in two species at the *lowest* concentration tested. This concentration was *less* than the existing AEL for the work place. Similarly, the human data (Bramwell *et al.*, 1963) do not support the existing exposure criteria. Moreover, some of the underlying fundamental human estimates for both GB and VX are not supported by the larger body of available data (Reutter and Wade, 1994; COT, 1997). Re-evaluation of the existing VX criteria was clearly necessary.

Careful review of the Bramwell *et al.* (1963) and Crook *et al.* (1983) studies revealed that the exposure paradigms were such that there was little confidence in the reported exposure concentrations. (They could have easily been lower or higher than stated.) Given this, the decision was made to develop the VX airborne exposure criteria based upon the estimated relative potency of VX to GB, using the GB criteria recently developed by Mioduszewski *et al.* (1998).

The potency factor that was chosen was 10, based upon estimated effective dosages for miosis or other mild effects (IDA, 1998). However, it was noted that, unlike GB, VX is a significant percutaneous vapor hazard. The estimated relative potency factor for threshold percutaneous effects is about 100. It was further noted that although Ct increases with exposure duration for GB, the limited data for VX tend to indicate the opposite.

Calculations are shown, for the Bramwell *et al.* (1963) and Crook *et al.* (1983) studies, as well as the estimated relative potency of VX as referenced to the GB criteria of Mioduszewski *et al.* (1998). The existing and recommended exposure criteria are given in Table 11.

**Table 11 Existing and Recommended Airborne Exposure Limits (AELs) for VX for Workers and the General Population**

| Criteria ($mg/m^3$) | | Application/ Scenario |
|---|---|---|
| **Existing** | **Recommended** | |
| **Occupational** | | |
| **0.00001** | **0.00001** | WPL (TWA) (8 hr/day, 40 hr wk) |
| **0.02** | **0.01** | IDLH (30 min) |
| **-----** | **0.00004** | STEL (15 min, 4x/day) |
| **General Population** | | |
| **0.000003** | **0.0000003** | GPL (TWA) (24 hr/day,7 days/wk) |
| **-----** | **0.0002** | AEGL-1 (30 min) |
| **-----** | **0.0001** | AEGL-1 (1 hr) |
| **-----** | **0.00003** | AEGL-1 (4 hr) |

**IDLH**: Immediately dangerous to life or health.
**STEL**: Short-term exposure limit.
**AEGL-1**: Acute exposure guideline, level 1 (limited to discomfort).
**TWA**: Time-weighted average

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## GLOSSARY/ACRONYM LIST

**Acute Exposure Guideline Level-1 (AEGL-1)**

The airborne concentration (expressed as ppm or $mg/m^3$) of a substance at or above which it is predicted that the general population, including "susceptible" but excluding "hypersusceptible" individuals, could experience notable discomfort. Airborne concentrations below the AEGL-1 represent exposure levels that may produce mild odor, taste or other sensory irritations. These guidelines are established by the National Advisory Committee to develop AEGLs under the authority of the Federal Advisory Committee Act (FACA) P.L. 92-463 of 1972. The AEGL-1 defined herein is applicable to 30-minute, 1-hour, and 4-hour exposures, as indicated.

**Airborne Exposure Limits (AELs)**

*Workplace*: Atmospheric concentration levels ($mg/m^3$) for the workplace, which represents conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse health effects, based upon 8 hr./day, 40 hr./week, for a working lifetime. Also called WPL.

*General Population*: Atmospheric concentration levels ($mg/m^3$) allowable for the general population (including sensitive subpopulations) for indefinite, unprotected lifetime exposure where no adverse health effects are expected as a result of exposure. Also called GPL.

**Acute Toxicity**

Toxic effects resulting from a single exposure to a toxicant and occurring within a 24 hr time-frame from the exposure period.

**Adverse Effect**

The biochemical change, functional impairment, or pathological lesion which impairs performance and reduces the ability of an organism to respond to additional challenge.

**Critical Effect**

The first adverse effect or its known precursor that occurs as dose rate increases.

**Delayed Toxicity**

Toxic effects not occurring until a lapse of some time after exposure; contrast with immediate toxicity.

**General Population Limit (GPL)**

Airborne exposure level (AEL) for long-term general population exposure expressed as an atmospheric concentration; see airborne exposure limits (AELs).

**Immediate Toxicity**

Toxic effects occurring or developing rapidly after a single exposure to a toxic substance; contrast with delayed toxicity.

**Immediately Dangerous to Life or Health (IDLH)**

The maximum airborne concentration from which, in the event of respirator failure, one could escape within 30 minutes without a respirator and without experiencing any escape-impairing (e.g. severe eye irritation) or irreversible health effects.

**Local versus Systemic Toxicity**

Local effects refer to those that occur at the site of entry (e.g., respiratory tract, eyes) of a toxicant into the body; systemic effects are those that are elicited after absorption and distribution of the toxicant from its entry point to a distant site.

**Lowest Observed Adverse Effect Level (LOAEL)**

The lowest exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between exposed population and its appropriate control group.

**No Observed Adverse Effect Level (NOAEL)**

The exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control; some effects may occur at this level, but they are not considered as adverse, nor precursors to specific adverse effects. In experimental studies in which several NOAELs are determined, the regulatory focus is primarily on the NOAEL seen at the highest dose. This leads to the common usage of the term NOAEL to mean the highest exposure without adverse effect.

**Reference Concentration (RfC)**

An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime. The EPA has adapted the reference dose method for oral exposures to set airborne exposure levels for health effects other than cancer.

**Severity**

The degree to which an effect changes and impairs the functional capacity of an organ system.

**Short-Term Exposure Limit (STEL)**

The concentration to which workers can be exposed continuously for a short period of time without suffering from: 1) irritation, 2) chronic or irreversible tissue damage, or 3) narcosis of sufficient degree to increase the likelihood of accidental injury, impair self-rescue or materially reduce work efficiency, and provided that the daily TLV-TWA s not exceeded. The STEL category of the TLV-TWA was developed by the American Conference of Governmental Industrial Hygienists (ACGIH) to define a 15-minute time weighted average (TWA) exposure which should not be exceed exceeded at any time during a workday even if the 8 hr TWA is within the threshold limit value (TLV) TWA. Exposures above the TLV-TWA up to the STEL should not be longer than 15 minutes and should not occur more than four times per day. There should be at least 60 minutes between successive exposures in this range.

**Threshold**

A dose level below which a response is unlikely, because homeostatic, compensatory and adaptive mechanisms in the cell or organism protect against toxic effects.

**Threshold Limit Value (TLV)**

A copyrighted term of the Committee of the American Conference of Governmental Industrial Hygienists (ACGIH) which refers to airborne concentrations of substances and represents conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse health effects. TLVs are based upon available information from industrial experience; from experimental human and animal studies; and when possible, from a combination of the three. The bases on which the values are established may differ from substance to substance; protection against impairment of health (those that shorten life expectancy, compromise physiological function, impair the capability for resisting other toxic or disease processes, or adversely affect reproductive function or developmental processes) may be a guiding factor for some whereas reasonable freedom from irritation, narcosis, nuisance, or other forms of stress may be the basis for others.

**Threshold Limit Value-Time Weighted Average (TLV-TWA)**

The time-weighted-average concentration for a normal 8-hour workday and a 40-hr workweek to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

**Threshold Limit Value - Ceiling (TLV-C)**

The concentration that should not be exceeded during any part of the working exposure. In conventional industrial hygiene practice if instantaneous monitoring is not feasible, then the TLV-C can be assessed by sampling over a 15-minute period except for those substances that may cause immediate irritation when exposures are short.

**Time Weighted Average (TWA)**

An averaging of exposure concentration over exposure time.

**Uncertainty Factor (UF)**

One of several factors used in operationally deriving the Reference Dose (RfD) or Reference Concentrations (RfC) from experimental data. UFs are intended to account for 1) the variation in sensitivity among the members of the general human population; 2) the uncertainty of extrapolating animal data to humans; 3) the uncertainty in extrapolating from data obtained in a study that is of less-than-lifetime exposure; 4) the uncertainty in using LOAEL data rather than NOAEL data; and 5) the inability of a single study to address adequately all possible adverse outcomes in man. the uncertainty arising when available data do not adequately address all possible adverse outcomes in man.

**Worker Population Limit (WPL)**

Airborne exposure level (AEL) for long-term worker population exposure expressed as an atmospheric concentration of substances and which represents conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse health effects; see airborne exposure limits (AELs).